

ÚSTAV LÉKAŘSKÉ BIOCHEMIE A LABORATORNÍ DIAGNOSTIKY 1. LF UK

# Selected examination methods in toxicology

---

*General Medicine*

Lenka Fialová

translated by Jan Pláteník



2018/2019

## 1. Poisons, toxic substances

Toxicology is an interdisciplinary field dealing with adverse (toxic) effects of exogenous chemical substances (xenobiotics) on living organism. Toxicology covers *two aspects: medical*, which focuses on the effects of poisons and optimal therapy of intoxications, and *analytical*, which deals with demonstration and determination of amount of usually unknown substance or mixture of substances and their metabolites in biological or non-biological material.

As *poisons (toxic, poisonous substances)* are denoted substances that following penetration to the organism are able to produce an injury even in a relatively small amount. Also drugs can act as poisons if overdosed.

Toxicologically significant substances include a wide array of substances with diverse physico-chemical properties:

- Drugs, addictive substances;
- volatile substances (e.g. ethanol, methanol, toluene, trichlorethylene);
- gases (e.g. carbon monoxide, hydrogen cyanide);
- inorganic acids and bases;
- metals (e.g. arsenic, lead, mercury).

## 2. Biological material

In toxicological analysis a diverse material is processed. It is important to send the material in sufficient amount so that in case of poisoning with an unknown toxin as many analyses as possible can be performed.

The usually examined kinds of materials are the following:

- ***Stomach content*** – at least 50 ml of vomits or the first portion of gastric lavage. Analysis of gastric content is useful in an early stage of intoxication when a yet unabsorbed substance can be present. In stomach content it is possible to find the the drugs in their original forms.
- ***Blood, or serum*** (10 ml) – level in blood is affected by the time passed since entry of the noxa (= harmful substance) to the body, rate of its absorption and excretion. Concentrations of poisons in blood are usually low. Blood is suitable especially for quantitative analysis.
- ***Urine*** – poison appears in urine later than in blood, but also persists longer, and so analysis of urine gains significance in later stages of intoxications. The noxious substance is often concentrated in urine, but it can be present as a range of its metabolites rather than in the original form. That is why analysis of urine can be more demanding. It is the most suitable material for capture and identification of poison. At least 100 ml is needed for analysis.
- ***Biological material obtained after death*** – contents of the gastrointestinal tract, tissue samples, and body fluids.
- ***Material secured in relation to intoxication*** – includes pills, liquids, injection syringes, and food remnants.
- ***Hair*** – accumulates certain substances, and so enables detection of drug abuse or chronic exposition.
- ***Exhaled breath*** – contains volatile substances.
- ***Saliva*** – utilized especially for rapid screening tests of drugs in controls of car drivers or employees on duty.
- ***Meconium*** – can be used in suspicion of drug abuse in a pregnant woman.

The samples of biological material should be transported in entirely clean vessels, free from traces of disinfectants and detergents. The vessels should be tightly closed; in particular in suspicion of poisoning with volatile substances or carbon monoxide the test tube must be filled completely and sealed tightly. Together with the biological material a form is send to the laboratory that should contain, in addition to the patient identification data, also information on the patient clinical status, suspicion of a specific poison, approximate time of poison ingestion and drug therapy before taking the sample.

### 3. Toxicological examination

The essential task in toxicological examination is detection of the presence of an unknown substance in biological material. Toxicological examination is provided for the needs of physicians in health care facilities, but can also be requested by authorities acting in criminal procedure.

#### 3.1. Indication for toxicological examination

The commonest *reasons for requesting a toxicological examination* are the following:

- Suspicion of intoxication with an unknown poison
- Differentiation of diagnosis of intoxication from other disease conditions – typically in case of patients hospitalized in intensive care units or resuscitation care departments; in these situations the emphasis is on rapid analysis.
- Intoxications in which there is a suspicion of a criminal act
- Demonstration of drug abuse and control of drug abstinence
- Monitoring of therapeutic efficacy and compliance

#### 3.2. Methods used in toxicology

Detection and identification of unknown toxin or toxins in biological material is very complex and analytical demanding process. Frequently it calls for a whole spectrum of analytical techniques.

The choice of analytical technique is affected by nature of the poison. The noxious substances can be classified to *volatile* and *extractive*; another division is to *inorganic* and *organic*. The extractive substances are further divided to acidic, basic or neutral according to the pH of medium to which they pass during extraction. The subsequent toxicological analysis relies mostly on various types of chromatographic methods, such as *thin layer chromatography* (TLC), *high performance liquid chromatography* (HPLC), and *gas chromatography* (GC), both the latter often coupled to *mass spectrometry* (MS). *Immunochemical methods* also play a significant role.

For estimation of volatile substances *gas chromatography* is used. Estimation of ethanol is of special importance, because of high frequency of examinations.

*Spectrophotometric methods* are employed for estimation of toxicologically significant derivatives of hemoglobin (carboxyhemoglobin, methemoglobin, sulfonhemoglobin, cyanmethemoglobin)<sup>1</sup>.

In analysis of inorganic substances further methods find usage, such as *atomic absorption spectrometry*, *neutron activation analysis* or *polarography*.

The general principles of techniques used in toxicology are explained in separate chapters.

#### 3.3. Workflow of toxicological analysis

The workflow of toxicological analysis includes several stages (fig. 1):

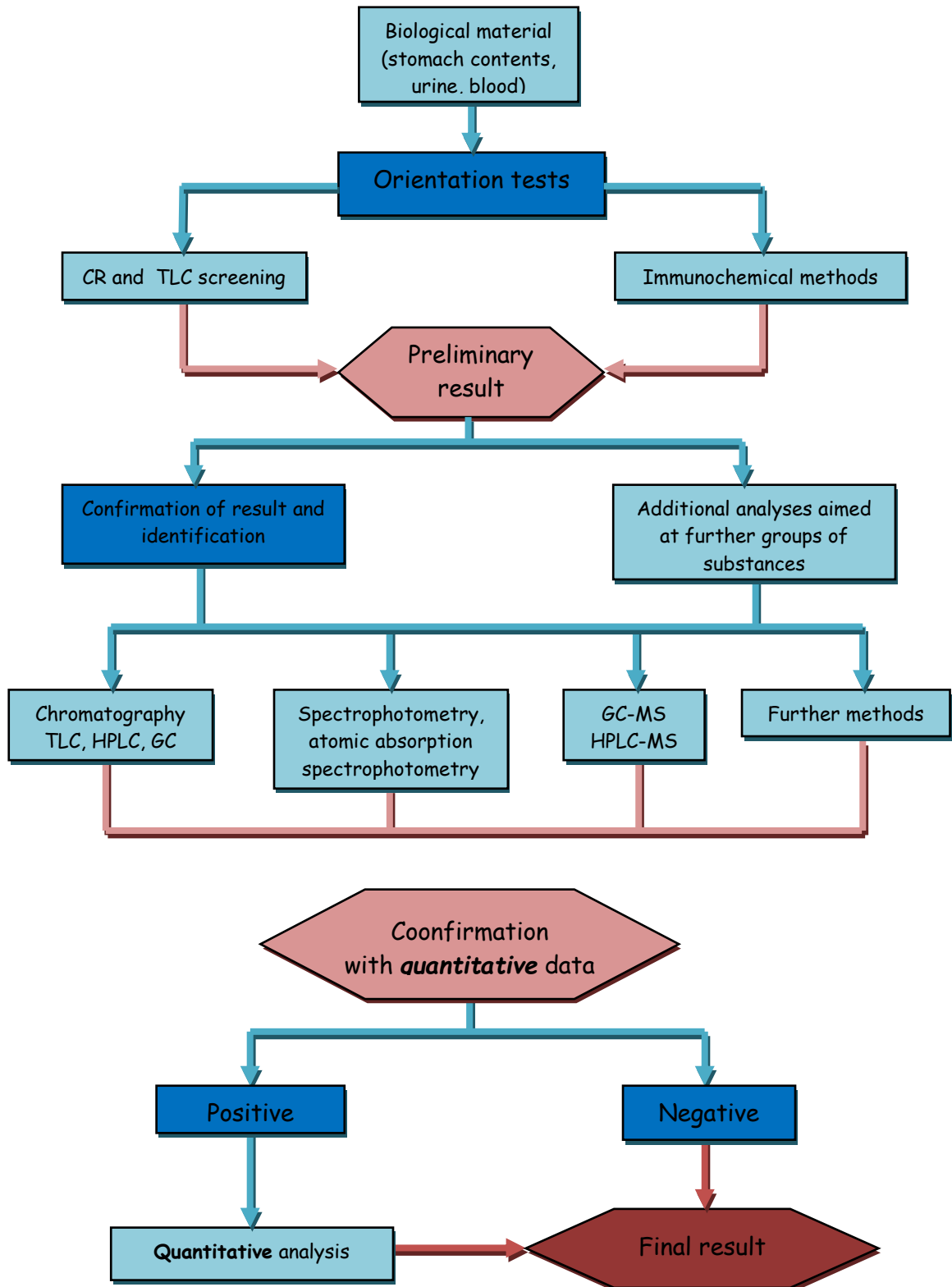
- *Screening* – based on immunochemical methods, thin-layer chromatography together with the system of colour reactions (CR), gas and liquid chromatography.
- *Demonstrations (identification) of a chemical entity – more sophisticated methods are* usually needed. The analysis employs liquid and gas chromatography with mass detection, thin-layer chromatography with various developing systems, sorbents and detection agents, and other methods.
- *Quantitative estimation of exogenous substance in biological material.*
- *Evaluation of findings by a toxicologist and physician.*

In the further explanation we will focus mostly on the first stage of toxicological analysis.

---

<sup>1</sup> See the text on practical lesson on hemoglobin and its derivatives.

Fig. 1 Scheme of the workflow in toxicological analysis



### 3.4. Screening

Screening of unknown noxa represents an introductory step of toxicological analysis. The results obtained with screening tests are rather preliminary in nature and it should be understood that the screening tests might not detect substances that are unexpected. The preliminary tests need not be specific but they should provide *sufficient sensitivity* so that the negative result can be considered reliable. In emergency cases (coma of unknown origin) rapidity of analysis is a necessary requirement. Occasionally, a preliminary result is sufficient to start a therapy while the examination can be later confirmed and elaborated with more specific and time-consuming methods.

In the screening of unknown substances especially immunochemical methods and thin-layer chromatography are used.

#### Immunochemical methods

This approach is suitable especially in the introductory phase of toxicological examination. Immunochemical detection of a drug is based on its reaction with a specific antibody. According to the antibody used, the detection can be limited to a group of immunochemically related substances (less specific antibody) or a single substance (highly specific antibody)<sup>2</sup>.

For the purpose of screening the immunochemical techniques are targeted at the groups of substances known as significant and often abused drugs (narcotic and psychotropic substances). The immunochemical group screening quickly sorts out suspect positive samples. An advantage is that the sample needs no processing before the test. Immunochemical analysers are available for these methods.

The tests falling to the category of *POCT* (Point Of Care Testing) are also based on immunochemistry. They enable a rapid analysis in the field or at the patient's bed outside laboratory with no requirements for instrumental equipment. The result is evaluated as positive or negative on the basis of accepted limits of positivity (the cut off values). In the form of diagnostic strips or plates they are used to test for drugs in urine. Recently also tests applicable to analysis of saliva or sweat have appeared.

POCT tests for drugs exist in various forms:

- *Paper strips* for qualitative or semiquantitative estimation of one analyte. Manipulation with the strip is very easy – the strip is dipped to urine for a few seconds, during which sample is absorbed to the strip. Then the urine migrates through the strip where the eventual noxa is demonstrated by means of immunochemistry.
- Strip tests for *one or several analytes* placed in a *protective frame*. In addition to the actual reagent zone the strip usually contains another control zone, which provides information whether the strip is functional. The examination lasts somewhat longer (several minutes) but the results are more reliable.
- *Testing vials* serve as a test and simultaneously for transport. On the side of the vial a testing area is located.

In this way it is currently possible to demonstrate the commonest used drugs – amphetamine, metamphetamine, benzodiazepines, cannabinoids, cocaine, opiates, phencyclidine, and tricyclic antidepressants.

One of the *principles* of estimation drugs in urine or saliva consists of a *competition between drug in the sample and a drug conjugate* that is immobilized in the strip detection zone. Both forms of the drug compete for a *limited amount of a specific antibody*.

Sample moves through the reaction zone containing the specific antibodies bound to colored microparticles, which are mobilized during the test. Both the sample components and the rehydrated labelled antibodies progress to the detection zone where they encounter the anchored drug conjugate.

---

<sup>2</sup> Detailed description of immunochemical methods can be found in the previously issued separate chapter on immunochemistry.

If the tested sample *contains a drug*, the binding sites of the color-labelled antibodies are saturated and cannot bind to the immobilized drug conjugate. The color of the detection zone remains unchanged and the result is interpreted as positive.

In the *absence of drug* in the tested sample the labelled antibodies are captured by the immobilized drug conjugate. A colored band is formed in the detection zone that means a negative result.

The results obtained with the tests described above represent only preliminary information that *must be confirmed by a more specific chemical method*.

## TLC/CR-screening

The principle of the method lies in detection of an unknown drug and/or its metabolites in biological material by means of several criteria, each of which enables classification of the detected drug or metabolite to certain group. An advantage of TLC screening is detection of a fairly wide range of substances and their metabolites and also ability to *resolve the particular components in analysis of mixtures*. It is a simple and inexpensive technique. Unlike the immunochemical methods, when the TLC is used a sample processing prior to the chromatography is necessary.

The TLC screening includes several steps. Each of them contributes to understanding of nature of the searched substance.

### *Extraction*

The searched substance is usually present in low levels in biological material. The purpose of extraction is concentration of the drug, which was originally in an aqueous phase (biological fluid), to an organic solvent, with a simultaneous removal of majority of ballast substances. Solubility in the organic phase is facilitated by pH adjustment of the examined material, which suppresses dissociation of acidic or basic functional groups. The approach called *fractional extraction* at various pH separates the extractive substances to three groups:

- *acidic*, which are extracted from biological material after its acidification;
- *basic*, which are obtained after alkalisation of biological material;
- *neutral*, which are extracted independently on pH.

The procedure of fractional extraction is following:

50 ml of analysed material is acidified with diluted HCl to pH 3 – 4 and repeatedly shaken with diethyl ether. The organic phase separates; the obtained extract contains substances acidic in nature (e.g. derivatives of salicylic acid) and part of the neutral substances. The separated aqueous phase remaining after the first extraction is alkalinized with sodium carbonate (pH 10 – 11) and again repeatedly shaken to diethyl ether. The second extract contains basic drugs (e.g. alkaloids, many analgesic drugs, sympatomimetics, tricyclic antidepressants) and part of the neutral substances (Fig. 2).

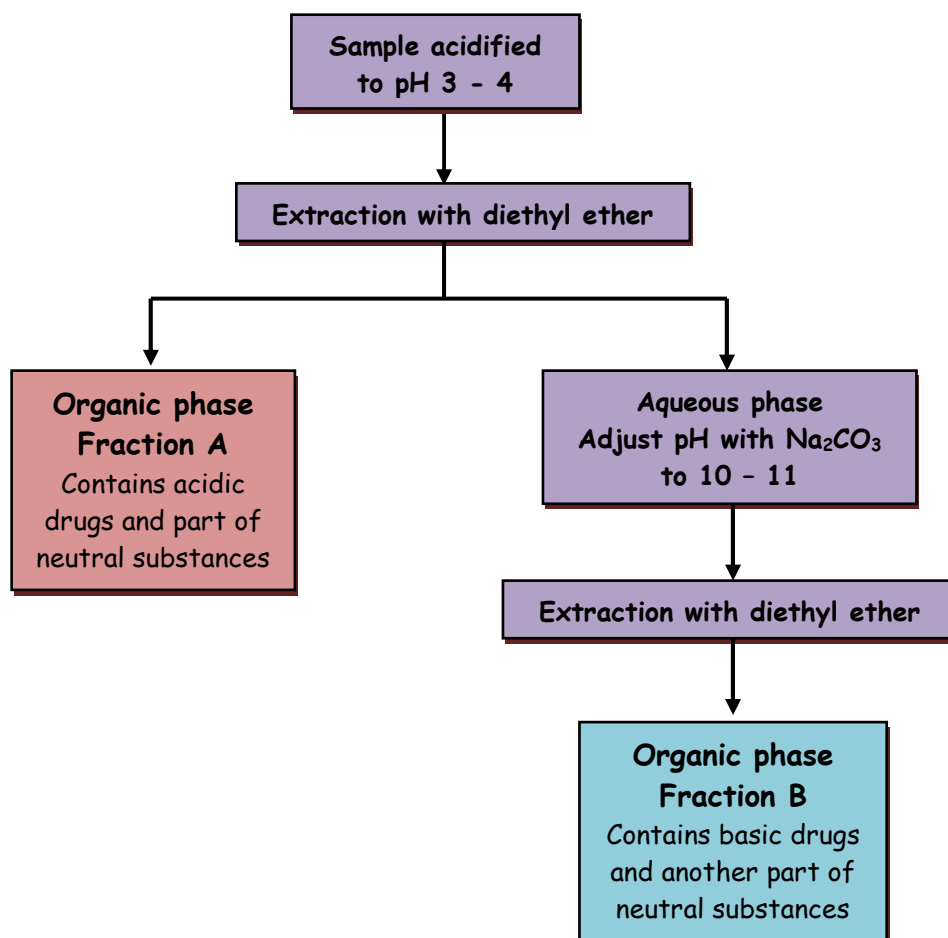
The procedure yields two extracts – *acidic extract (Ex<sub>A</sub>) and basic (alkaline) extract (Ex<sub>B</sub>)*.

Finding of a substance in the acidic or alkaline extract, or in both (neutral substances) is only a first step in its identification. The extraction of unknown substance prior to analysis is not required only in some immunochemical methods and in some cases of HPLC.

If presence of metabolites in a conjugate form (most often with glucuronic acid) is expected in the sample, the extraction is preceded by their hydrolytic cleavage.

Another, more ecological way of extraction of drugs from biological material, in which consumption of organic solvents is low, is based on a *solid phase extraction*. The principle is column chromatography in various modifications. A suitable sorbent (silicagel, aluminium oxide) is packed to the columns. During passage through the column the searched substance or group of substances is trapped by the sorbent. Alternatively, the ballast substances are bound to the column whereas the drug passes through without binding.

Fig. 2 Scheme of fractional extraction






































### 3.5. CR (color reactions) screening




The actual chromatography can be preceded with a **CR-screening**. It employs reactions of many drugs with inorganic (acids, salts of heavy metals) or organic reagents, in which characteristic colored products are formed.

The CR-screening is performed in a form of **dot reactions**, where drops of the analysed sample or its extract are combined with various reagents in certain order. On the basis of colors produced with the group reagents and further auxiliary reagents the drugs are classified to several groups – classes of colored reactions. The first positive reaction with the given order of reagents is decisive for class determination (Fig. 3).

The CR-screening represents a typical screening method; its usage can exclude or suggest presence of certain drug in the analysed sample. A group of structurally related drugs can be identified in this way. The method is **not suitable for detection of drugs in mixtures**. Examination of biological material **only by CR-screening** has a limited significance.

Fig. 3 CR-screening and division of drugs to classes of colored reactions<sup>3</sup>

Class	Reagents						
	Marquis	p-DMAB	Ce(SO <sub>4</sub> ) <sub>2</sub>	AuCl <sub>3</sub>	Drag.	HNO <sub>3</sub>	FeCl <sub>3</sub>
I							
II							
III							
IV							
V							

 Reaction characteristic for group  
 Reaction negative  
 Reaction auxiliary and differentiating

As the usable biological material the stomach content seems to be most likely. In the stomach content an original form of the drug can be expected and the results are not affected by presence of certain interfering substances. Analysis of urine is complicated by the presence of ballast substances and metabolites, which can react differently from the original substance.

However, if the CR-screening is *combined with TLC*, it provides quick and cheap preliminary information that guides further analysis. The CR-screening results suggest which colored reactions should be used for detection in TLC analysis.

<sup>3</sup> Composition of the reagents is included in the instructions for practical lesson ( p-DMAB - p-dimethylaminobenzaldehyde).

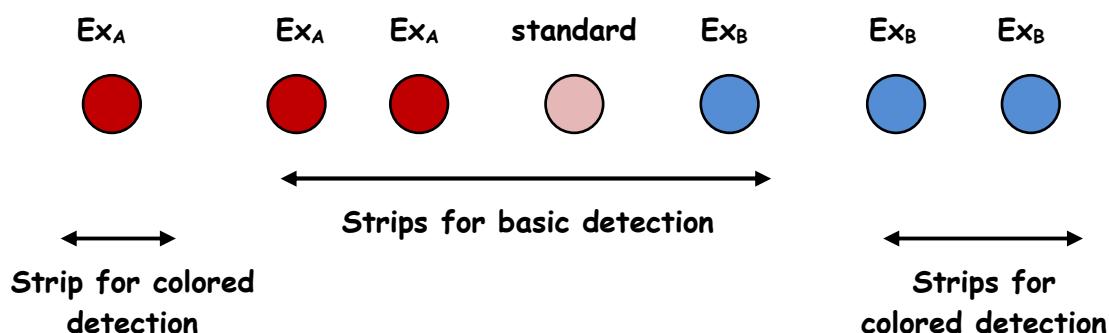


### 3.6. Thin-layer chromatography

The analytical system of thin-layer chromatography contains a **stationary phase** that is usually silica gel (hydrated silicic acid). The mobile phase is formed by a mixture of solvents of various polarities; acetic acid or ammonia is added to suppress eventual dissociation of the analysed<sup>4</sup>.

On the thin layer *samples of acidic and basic extracts* are applied into several starts that are designed for basic and colored detection, respectively (Fig. 4). Simultaneously with the unknown samples a standard mixture is analysed whose components are chosen so that they distribute along the whole length of the chromatogram<sup>5</sup>. *Development of chromatogram* takes place upwardly in a closed chromatographic chamber. The chromatogram is then removed, dried and subjected to procedures aimed at detection of the spots.

Fig. 4 Example of application of starts for TLC/CR-screening



For *detection of the spots*, whose essence is visualization of colorless substances, the following basic procedures are:

- **Detection in UV light** at 254 nm and 366 nm;
  - Demonstration of fluorescing substances by visual inspection of the chromatogram;
- **Visual detection**
  - Basic colored detection by spraying the strips with  $EX_A$  as well as  $EX_B$  with universal Dragendorff reagent;
  - Colored detection with further specific organic or inorganic reagents, chosen according to the results of CR-screening.

#### Evaluation of the finding

Several criteria are evaluated:

- **Classification of the drug to extraction group**
  - Substance found only in one extract
    - in  $EX_A$  → substance is a weak acid (or neutral in low concentration),
    - in  $EX_B$  → the found substance is a base,
  - Substance is found in both extracts  $EX_A$  and  $EX_B$  → the detected substance is neutral.
- **Position of the spots on the chromatogram**

The position is related to the nearest standard from the simultaneously run standard mixture and is expressed with the symbol  $R_s$ . For example, position of a spot located in the region between atropine and codeine is marked as AtC (fig. 5).
- **Reaction ability of substances during detection**

Fluorescence and color of the spots including their tints, color intensity and course of the reaction (rate of appearance, color deepening or fading) are evaluated.

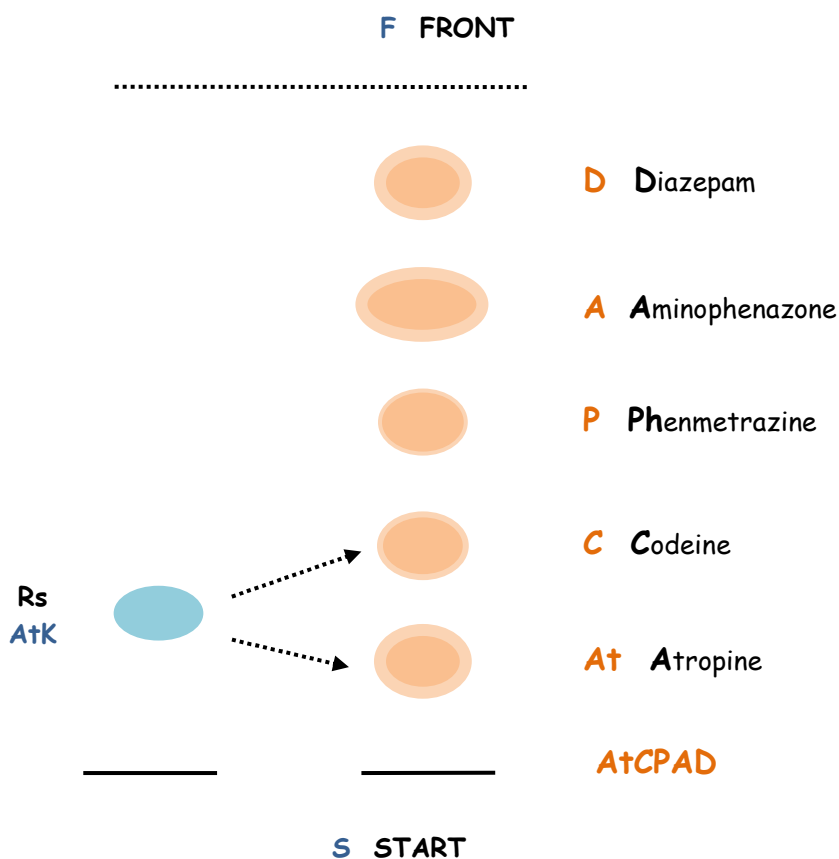
<sup>4</sup> The basic mobile phase consists of mixture of ethyl acetate – ethanol – ammonia in ratio 36 ml : 2 ml : 2 ml.

<sup>5</sup> Atropine – codeine – phenmetrazine – aminophenazone – diazepam, i.e. AtCPAD

The evaluation of findings in TLC/CR screening should lead to a narrow range of substances that could be present in the sample. The next step is their *identification*, in which the properties of searched substance are compared with another one which supposedly is identical (standard). In chromatographic identification the sample is analysed together with the standard and the analysis is repeated in at least 2-3 different suitable mobile phases and auxiliary detection procedures are applied. The identity of the searched substance with the standard can be assumed if both display the same value of  $R_F$  as well as the same reactivity in detection.

In case the TLC identification is not successful, it is possible to scratch the spot out of the chromatogram, extract the substance and continue in analysis by more sophisticated techniques.

**Fig. 5 Standard AtCPAD mixture and determination of  $R_s$**



#### 4. Biotransformation of drugs

Majority of exogenous substances in the body undergoes *biotransformation*, in which metabolites originate. Especially lipophilic substances are intensively metabolized. Their conversion increases polarity and enables excretion of the resulting metabolites to urine.

It often happens that the original form of the drug is not present in the sample. Then *demonstration of metabolites* gains importance. It is needed especially in drugs with a short half-life, in which the blood sample was taken following metabolism of the original drug. From this reason, in evaluation of the toxicological findings the following situations should be taken to the account:

- drug is present in its original unchanged form;
- drug is demonstrated in original form together with a metabolite;
- metabolites prevail.

The conversion of the original drug to metabolites complicates the toxicological analysis. From the analytical point of view, it is important to consider that biotransformation leads to

metabolites having properties different from the original drug. For example, presence of ionizable groups in the molecule of metabolite causes that metabolite passes to different extraction fraction than the original form. Metabolites are usually more polar than the original structure and therefore tend to migrate less in TLC. A common biotransformation process is conjugation of the xenobiotic with various endogenous polar compounds, such as glucuronic acid, glycine, and sulfuric acid.

#### 4.1. Examples of biotransformation

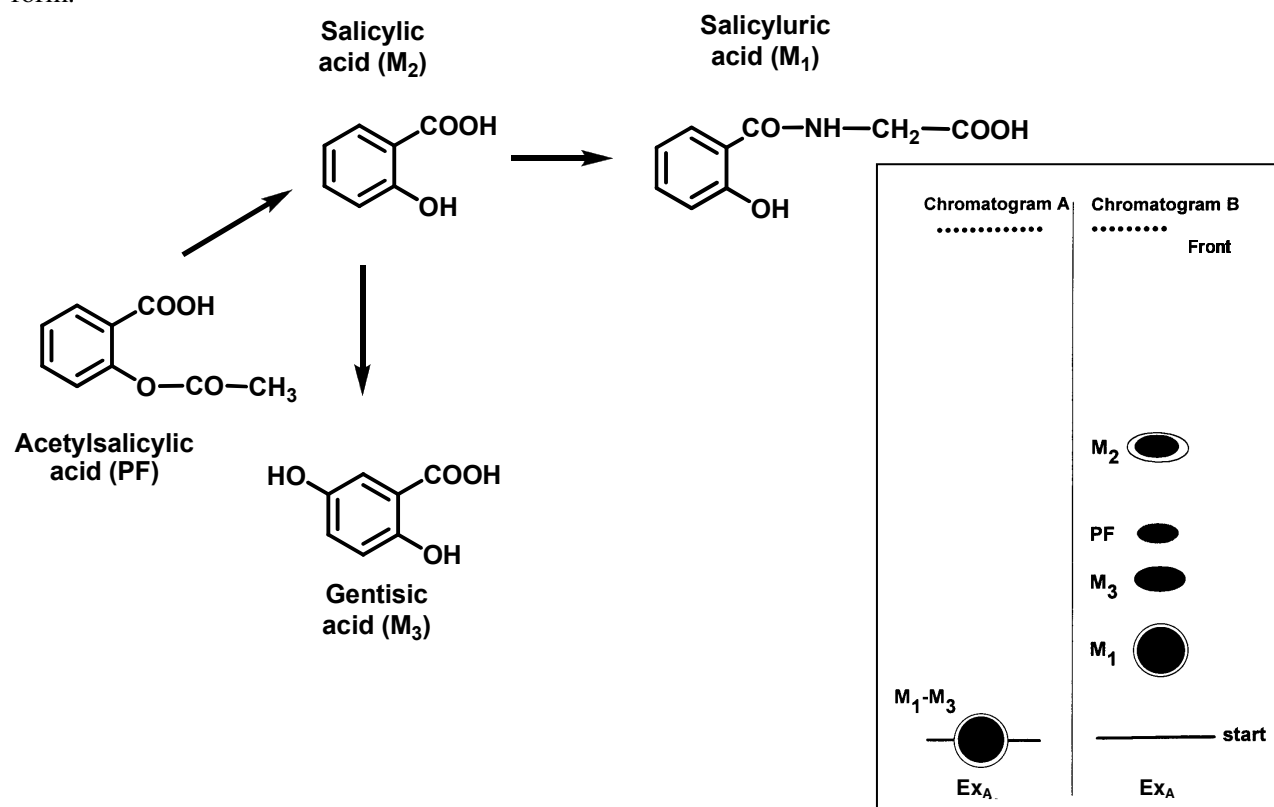
##### Biotransformation of acetylsalicylic acid

Acetylsalicylic acid is widely used analgesic and antipyretic remedy<sup>6</sup>. In toxicological analytics is encountered in intoxications of children or suicidal attempts of adults, where it is usually detected together with other drugs, found in the combined analgesic-antipyretic mixtures.

The core reaction in biotransformation of acetylsalicylic acid is its cleavage to acetic acid and salicylic acid ( $M_2$ ). Part of the salicylic acid is excreted as such, whereas another part appears in a conjugated form with glycine as salicyluric acid ( $M_1$ ). Yet another portion of salicylic acid is hydroxylated to gentisic acid ( $M_3$ ) (Fig. 6).

Hydroxylation and conjugation of the original drug increases polarity of the resulting molecules. As a consequence mobilities of the products in chromatographic separation decrease. In TLC/CR-screening analysis with the basic developing system the acetylsalicylic acid and their metabolites remain on the start. In order to increase the mobility and separation of metabolites a different composition of the mobile phase must be chosen. Detection of the spots can employ either ferric chloride that forms a colored complex (violet-red) with derivatives of salicylic acid or UV light (254 nm) (Fig. 6).

**Fig. 6 Biotransformation of acetylsalicylic acid and its detection in urine with TLC chromatography on SILUFOL (chromatogram A – basic mobile phase, chromatogram B – mobile phase of composition cyclohexane – tetrachlormethane – diethyl ether – acetic acid in ratio 10:10:10:5; detection of chromatograms A and B with ferric chloride). M – metabolites, PF – original form.**



<sup>6</sup> Analgesic drugs suppress pain (painkillers), antipyretic drugs lower body temperature in fever.

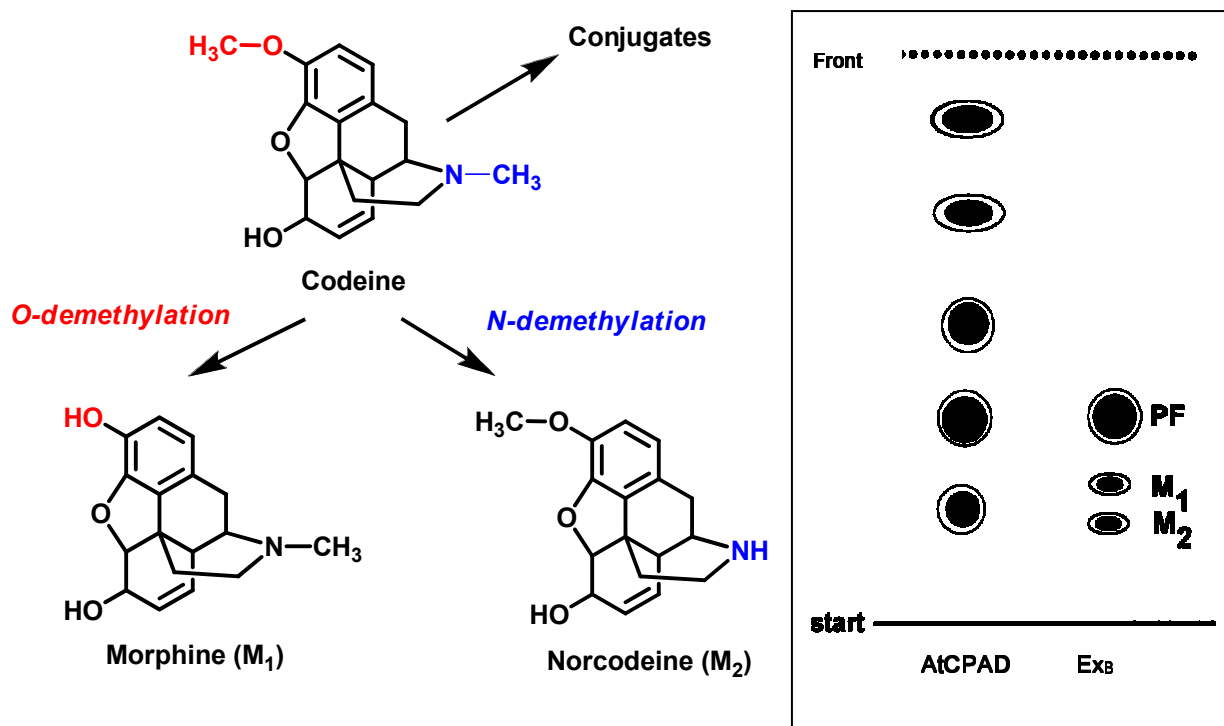
## Biotransformation of codeine

Codeine is a remedy used to suppress cough; in addition it is a part of analgesic combinations. It is one of the substances abused by drug addicts.

Interestingly enough, in biotransformation of codeine one of the metabolites is morphine. It is a serious fact that needs to be taken into account in interpretation of toxicological analysis. Morphine ( $M_1$ ) originated through demethylation reaction on oxygen, called O-demethylation. Codeine in the body undergoes also another type of demethylation on nitrogen - N-demethylation, which yields norcodeine ( $M_2$ ) (Fig. 7).

In the screening analysis codeine is found in alkaline extract from urine in the original form accompanied by small amount of metabolites with lower  $R_f$  (Fig. 7). However, the major part of codeine is excreted as conjugate with glucuronic acid, hence the sample should be also analysed after hydrolysis.

**Fig. 7 Biotransformation of codeine to morphine and norcodeine and detection in alkaline extract of urine with TLC chromatography on SILUFOL in the basic mobile phase, detected with Dragendorff reagent ( $M_1$  – morphine,  $M_2$  – norcodeine, PF-original form)**



## 5. Ethanol in toxicology

Estimation of ethanol in biological material is one of the commonest requests for examination in toxicological laboratory.

The main pharmacological action of ethanol takes place in the CNS. Low doses induce euphoria and excitation. Ethanol at higher doses has narcotic effects; it can cause unconsciousness and even death in the most severe cases.

The ingested alcohol is readily absorbed in the digestive tract. It is distributed to all organs. The metabolism of ethanol takes place mostly in the liver, where it is oxidized by means of the enzyme alcohol dehydrogenase to acetaldehyde, which is further converted to acetic acid. About 90 – 95 % of ethanol is oxidized; the remaining part is excreted unchanged to breath and urine.

## 5.1. Estimation of ethanol

There are various methods how to find out that a person has drunk an alcoholic beverage or his behavior is under the influence of ethanol. In many cases it is sufficient to perform a **screening test for ethanol in exhaled breath**. In case of a positive result a **demonstration of ethanol in blood** follows. A blood sample is taken from the examined person and analyzed in a specialized laboratory using more accurate methods.

### 5.1.1. Preliminary estimation of ethanol in exhaled breath

Breath analyzers or detection tubings are available for examination of ethanol in exhaled breath. A negative breath test is sufficient as a proof that the breath does not contain ethanol.

#### Breath analyzers

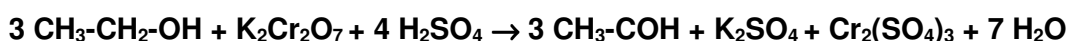
Some breath analyzers utilize for determination of the presence of ethanol in exhaled breath sensitive *electrochemical detectors* on the basis of ion mobility spectrometry (IMS). The exhaled breath is exposed to radiation effect of americium ( $^{241}\text{Am}$ ), which causes its ionization. In an electric field the ions move with a characteristic speed that is recorded in a form of impulses and evaluated with a special software.

Other types of breath analyzers, which serve only as screening devices, are *semiconductor sensors*.

#### Detection tubings

In the past the ethanol in exhaled breath was detected by means of detection tubings. The test for ethanol utilized a reduction of some substances ( $\text{K}_2\text{Cr}_2\text{O}_7$ ,  $\text{KMnO}_4$ ) with ethanol, seen as a color change.

Reaction with potassium dichromate is used in the detection tubings **Altest**. The tubing is filled with silicate grains coated with a thin layer of potassium dichromate and sulfuric acid. The air containing ethanol vapors reduces the indication mixture. Reduction changes yellow  $\text{K}_2\text{Cr}_2\text{O}_7$  to green  $\text{Cr}_2(\text{SO}_4)_3$ . Ethanol oxidizes to acetaldehyde. The reaction is unspecific, and can be turned positive also e.g. by fruit, candy, and mouth wash.



According to the height of the green layer it is possible to roughly estimate the concentration of ethanol in blood. The detection tubings could reveal persons with concentration of ethanol in blood roughly above 0.4 g/kg.

### 5.1.2. Estimation of ethanol in blood

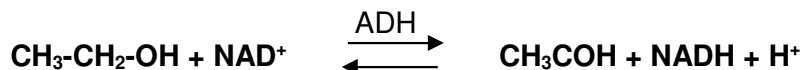
Unlike the screening tests above, the analytical methods for estimation of ethanol concentration in blood are very accurate and their results are decisive from the forensic point of view. For the forensic purpose the estimation of ethanol must be performed in every sample with two independent methods based on different principles.

*Gas chromatography* is considered as an objective method acceptable for the court. It enables a qualitatively specific and quantitative estimation of concentration of ethanol in the blood. One of its advantages is that ethanol is conclusively differentiated from other volatile substances such as methanol, acetone or toluene.

For the quantitative evaluation a method of internal standard is used. As an internal standard a substance that is absent from the sample and unreactive with any sample component can be used.

In the analysis a sample of biological material is mixed with the internal standard in an exact volume ratio. In the same way an ethanol solution of known concentration (standard) is prepared. Then the mixtures are evaluated with of the gas chromatograph.

An **enzymatic method** can be used for estimation of ethanol as well. Ethanol is oxidized by  $\text{NAD}^+$  with catalytic action of the enzyme alcohol dehydrogenase (ADH) to acetaldehyde. The amount of reduced NADH is proportional to the amount of ethanol in the sample. Absorbance of NADH is measured in UV region at 340 nm (the Warburg optical test). This method is not absolutely specific for ethanol, but it can be used for clinical purposes.



In the past also a less specific titration **Widmark test** was used. Its principle was based on oxidation of ethanol to acetic acid with potassium dichromate  $\text{K}_2\text{Cr}_2\text{O}_7$ . The excess of unreacted dichromate was estimated iodometrically after addition of potassium iodide.

Concentration of ethanol in blood is expressed in SI units g/kg, which numerically correspond to ‰. For medical purpose the concentration is given in mmol/l (1 g/kg = 21.71 mmol/l). In the gas chromatography analysis the alcohol in blood is considered positive from the forensic point of view if a value 0.21 g/kg is found or more<sup>7</sup>.

#### Used literature:

- Babjuk J. a kol.: Bioanalytika léků. Avicenum, Praha, 1990.
- Balíková M.: Toxikologické analýsy pro potřeby klinické a soudní. Lékařské listy, 26.7. 2001, str. 6-8,
- Balíková M.: Forenzní a klinická toxikologie. Galén, Praha 2004.
- Chundela B. a kol.: Vybrané kapitoly z toxikologie. Institut pro další vzdělávání středních zdravotnických pracovníků, Brno 1986.
- Kraml J. a kolektiv: Návody k praktickým cvičením z lékařské chemie a biochemie. Karolinum, Praha 1999.
- Prokeš J. a kol.: Základy toxikologie I. Karolinum, Praha, 1997.
- Prokeš J. a kol.: Základy toxikologie II. Karolinum, Praha, 1998.
- Prokeš J. a kol.: Základy toxikologie. Galén, Karolinum, Praha 2005.
- Samková H., Pivnička J.: Toxikologie – pomocník, svědek a důkazový materiál. Lékařské listy, 11.7. 2002, str. 5 – 7.
- Racek J. a kolektiv: Klinická biochemie. Galén-Karolinum, 1999.
- Šprongl L.: Možnosti ultrarychlé toxikologické diagnostiky u akutních příhod toxikomanů. Postgraduální medicína, 564-567.
- Štípek St.: Stručná toxikologie. Medprint, Praha 1997.
- Thomas I.: Clinical laboratory diagnostics. TH-Book, Frankfurt/Main, 1998
- Večerková J.: Postupy při záchytu a identifikaci léčiv a jejich metabolitů v biologickém materiálu pomocí chromatografie na tenkých vrstvách, SNP, Praha, 1983
- Večerková J.: Léčiva v biologickém materiálu. In: Materia pharmaceutica 5. Ed. Horáková O. a kol., Osveta 1987.
- Zedníková K., Ondra P.: Toxikologická vyšetření - možnosti, úskalí a pozitivní nálezy. Lékařské listy, 26.1. 2001, str. 9 – 12.
- Zima T. a kol.: Laboratorní diagnostika. Galén, Praha, 2002.

<sup>7</sup> Value below 0.20 g/kg is considered inconclusive. A possibility of laboratory error is taken into account.