NEWIP | NIGHTLIFE EMPOWERMENT & WELL-BEING IMPLEMENTATION PROJECT

TED1

Guidelines for Drug Checking Methodology

This Guidelines for Drug Checking Methodology arises from the Nightlife Empowerment & Well-being Implementation Project which has received funding from the European Union, in the framework of the Health Programme

Executive Agency for Health and Consumers

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Contents

01
Introduction

Harm reduction has become the focus of public health initiatives and therapeutic interventions in the management of drug addiction over the last 20 years. In the last decade these approaches have been extended to include recreational drug use. Most harm reduction initiatives are focused on providing information to users about risks and ways to minimize them. The concept of providing illicit drug users with quality assessment of their chosen drug is one possible harm reduction intervention that, until recently, has received little attention. In response to well-publicized 'ecstasy'-related deaths, non-governmental organizations (NGOs) in some European countries and the United States are offering a "pill testing service" for users (Winstock et al. 2002).

For many users Drug Checking is often the first point of contact with the social support system. Facilitating access for this target group through Drug Checking services legitimizes the costs associated with the analysis of drug specimens. Furthermore, by offering these consumers a specific service (substance analysis) it is easier to motivate them to participate in a consultation or a counselling session (Hungerbuehler et al. 2011). Drug Checking can be done in a drug counselling centre and also onsite e.g. at parties, raves, and festivals.

This document compiles a list of several techniques currently used in Drug Checking programs, its aims are:

- to help services that might be interested in implementing a Drug Checking Program.
- to provide complementary information to services that are actually proposing such a service.
- to standardize the methodologies developed in Europe, making the results comparable and serviceable for extended application.

For these reasons, this document is focused on concrete technical information, including benefits and limitations, targeting the Drug Program managers or, directly, the Programs themselves.

If more specific information is needed, contact persons are mentioned for each technique.

The document will constitute part of a larger standard "Implementation of Drug Checking services standard", which includes complementary information such as "History of Drug Checking", "Legal framework", and "Evaluation of Drug Checking services".

02

Thin Layer Chromatography (TLC)

There are various Thin Layer Chromatography (TLC) methods.

The two main ones used in Europe are the TOXILAB® and the TLC developed by Energy Control (EC) in Spain. Each of them has its advantages and disadvantages.

For example, whilst the TOXILAB® is easy to buy, set up, and use, it involves the handling of formaldehyde and sulphuric acid which require special security measures.

On the other hand, the EC method is particularly safe it but needs a stock of pure drug standards to start the analysis, and only a few official laboratories, subject to strict rules, are authorized to use these standards. The TLC technique from EC has been set up in an IMIM laboratory thanks to the collaboration established between IMIM and EC. IMIM is authorized to manipulate the standards of most illegal substances. From these standards the various TLC systems were designed. Once the TLC has been set up in a laboratory, it can work without reference standards by using RF-values (retardation factor) previously calculated in the laboratory. EC strongly recommends that before starting with this type of TLC collaboration with a laboratory is established. With this collaboration it is possible to have access to the standards for setting up the TLC and identifying new substances in a constantly changing drug-market.

Since 2000, the EC Spanish project has offered a Drug Checking Service with colorimetric tests, TLC, and other chromatographic techniques (GC and HPLC) coupled to mass spectrometry. The EC Drug checking Service is performed through an agreement with the Human Pharmacology and Clinical Neurosciences Research Group at IMIM-Hospital del Mar Research Institute (IMIM). This collaboration permits TLC methodology validation, access to more sophisticated analytical techniques for the specific identification of new adulterants and drugs, and evaluation of the pharmaco-toxicological relevance of some findings in drug specimens.

Introduction to the technique

Thin layer chromatography is a useful method for the screening, separation, and identification of the pure components in a mixture.

Separation is possible due to the difference in the interaction of the molecules that are present in the mixture with a mobile phase, (typically a mixture of solvents with different polarities) and a stationary phase (typically silica gel).

The number of components in the sample can be ascertained by TLC. The difference in the mobility of components in the stationary phase permits their separation and identification.

A semi-quantitative estimation of the concentration of the components is possible if adequate reference standards are analysed, in parallel, in the

same run. See the Methods section of EC for which type of standards are recommended to set up a TLC method. TLC can also provide valuable information before proceeding on to more instrumented approaches.

Clues to the chemical structure of a component can be obtained by noting its mobility on the TLC plate in different solvent systems and the reactions to a variety of chemical reagents.

A given compound is identified by its RF value. This can be determined for each component by dividing the distance it travelled by the distance the solvent front travelled, using the initial spotting site as reference. Reagents applied to the plate, or some physical properties of the components (UV absorption or fluorescence emission) once the chromatographic run is finished (by predefining a distance to be travelled by the mobile phase from the initial spot), allow the identification of components. The specificity of TLC is greatly increased by using multiple solvent systems of different polarities or pH.

SUMMARY OF ANALYTICAL CHARACTERISTICS

Benefits: relatively cheap, quick, and easily available. Small sample amounts (5 mg) are required.

Disadvantages: limited accuracy in identification, it is better to have TLC validated with a high reliability technique such as GC/MS. For identifying new substances TLC has to be complemented with GC/MS or LC/MS. Quantification is difficult to perform.

Total cost for the technique:

Energy Control: 3,000-4,000 €

Toxilab: 6,500 €

Yearly Running Costs: 500-2,000 €, depending on the number of samples analysed per year and the TLC method used.

Cost per analysis:

Energy Control: 1-3 €.

Toxilab: 65 €

Reliability of results: medium, only qualitative results. For quantitative data an expensive instrumental set up is necessary.

Identification of substances: low specificity, identification requires comparison with RF-values or reference substances. The RF-values depend on the humidity and the temperature of the environment, so if TLC is performed in festivals with extreme conditions it is necessary to calibrate the RF-values accordingly. Calibration can be carried out with legal substances such as ephedrine, caffeine, paracetamol, and lidocaine.

Availability of the instrument: not many instrumental requirements. All the equipment needed for TLC can fit on a table.

Duration of analysis: approximately 30 minutes, several analyses in parallel are possible.

Staff requirements: a chemist or pharmacologist is required to set up the method To perform TLC, however, a technical degree is not required. TLC can be performed with 1 or 2 technical staff trained in the analytical procedures and basic pharmacology/toxicology of drugs.

Suitable for onsite Drug Checking: only when minimal environmental conditions of ventilation and hygiene are present due to the solvent vapours. If the intervention is in a closed space an extraction capacity of 1000 cubic meters per hour is recommended, it is, however, always better to perform TLC in an open space without wind and sand.

Implementation & organizational recommendations for the technique: TLC is an inexpensive technique compared with other more instrumented ones (GC-MS, NMR, etc.). It is also easy to apply, for this reason it is suitable for both recreational settings and stationary Drug Checking Services.

The interpretation of results is difficult and well-trained technical staff are required.

Due to the fact that there are compounds that TLC cannot identify, or the existence of new substances from which standards are not yet available, TLC needs to be complemented by other techniques such as GC-MS or NMR. Within this context, the agreement of EC with IMIM is essential in order to have access to GC-MS and LC-MS.

It is necessary to take into consideration that, for a number of reasons, collaboration with a laboratory could be difficult: - there are not many laboratories carrying out drug analysis, and sometimes the nearest one is in another city.

- bringing the samples to the laboratory requires, in some countries, special authorization to transport illegal substances.

- not all laboratories are willing to collaborate with harm reduction services

- these complementary analyses have a cost (between 30 and 200 € for each sample) which has to be added to the TLC expense.

- there may be a relatively long delay in obtaining results.

It is also necessary to be aware of the waste residues generated which need to be treated by a specialized recycling company.

To develop its activities EC is financed by local and national governments. The number of samples analyzed depends only on demand and not on budgetary limitations. Government funding is essential to maintain this type of service. A factsheet designed for decision makers, offering a concise overview of current Drug Checking activities in Europe, is available on TEDI's website.

MATERIALS

Material for	Energy Control[1]	TOXILAB® system
Operator safety	Latex or nitrile gloves Protective eyeglasses Proper clothing	Latex or nitrile gloves Protective eyeglasses Proper clothing
Preparation of the samples	Watch glass or pestle for crushing the sample Spatulas Vials or eppendorfs micropipettes or glass/plastic Pasteur pipettes for dissolving samples Agitator vortex	Watch glass or pestle for crushing the sample Spatulas TOXI-TUBES A Distilled water Centrifuge TOXI-DISCS Pasteur pipettes Heather plaque

[1] The material used by EC is current TLC material available from any supplier of laboratory material.

Developing TLC	▲ Filter paper or some other suitable absorbent paper should line the back inside wall of the tank at a height greater than the plate. ▲ Thin layer plates- silica gel (250 micrometers) coated glass or aluminum plates with fluorescent indicator. ▲ Solvent tank: Any covered glass container with a level bottom. ▲ Rectangular tanks are most common. The recommended size is: 20 x 10 x 6 cm. ▲ Drug standards dissolved in methanol[2] ▲ Glass vials: 5-10 ml for standards ▲ Capillary tubes	▲ TOXI A-PLUS system (includes the solvent tanks and the plates)
Solvents	▲ Methanol ▲ Ammonia solution (25%) ▲ Acetone ▲ Test tube or flask to measure the solvents ▲ Funnel ▲ Pyrex jars or similar: 1 L, 0,5 L, 0,25 L	▲ Methanol ▲ Ammonia solution (25%) ▲ Ethyl acetate ▲ Test tube or flask to measure the solvents ▲ Funnel ▲ Pyrex jars or similar: 1 L, 0,5 L, 0,25 L
Visualization	▲ UV chamber for operator safety ▲ Short wave UV light source (254 nm) ▲ Colorimetric tests: Marquis & p-DMAB-TS[3]	▲ UV lamp ▲ Sulfurique acid ▲ Formaldehyde ▲ Distilled water ▲ Draggendorf reagent

[2] See Methods section for which type of standards are recommended to set up a TLC method.

[3] For more detailed information see Annex II of colorimetric tests.

METHODS (For EC method)

Preparing the glass container

1. Cut blotting paper and place it on the wall of the container.

2. Preparing the solvent:

- Phenetylamines (amphetamine, MDMA, 2C-B, etc.), Indolamines (LSD, DMT, etc.) derivatives and opiates system (methanol:NH_4OH (25% ammonia solution), 100:2.5 v/v).
- Cocaine system (acetone 100%) for the other big container.
- Cocaine and Ketamine system (methanol) for the small container.

It is recommended to use the three solvent systems for unknown substances. A colorimetric test is useful in order to obtain an initial idea of which kind of substance is present and the corresponding TLC system to be employed.

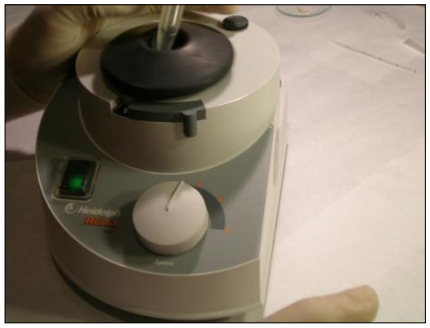
The following table describes which substances are detected in which solvent:

Solvent	Substances detected
Methanol/ammonia 100:2,5 system	MDMA, amphetamine, mCPP, metoclopramide, caffeine, acetaminophen, procaine, 2C-B, DMT, LSD, heroin, DXM, codeine.
Acetone 100%	cocaine, lidocaine, procaine, tetracaine, benzocaine, acetaminophen, phenacetine, caffeine, mephedrone.
Methanol	cocaine, levamisole, lidocaine, procaine, tetracaine, benzocaine, acetaminophen, caffeine, ketamine.

The three solvents described above can identify most substances, although some other solvent mixes may be used for specific cases such as the identification of tryptamines.

3. Add the solvent to the container so that it soaks the blotting paper. The paper should be lying against the glass wall. Cover the container. Leave it for 15-30 minutes, so the solvent vapours can saturate the glass container.

Preparing the samples

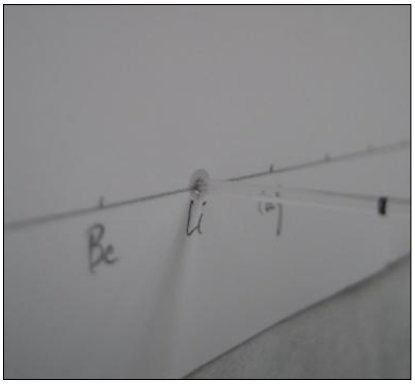


1. Place 5mg of the sample (exact weight is not necessary) in an eppendorf. With LSD blotters or other substances use ¼ of the blotter. In case of LSD microdots, use ¼ of the pulverized microdot.
2. Add 0.5 ml of methanol to the sample in the eppendorf.
3. Shake the sample vigorously for a few seconds (manually or with a shaker).

Preparing the TLC plate

1. Cut the thin layer plate: 10 cm width, and as high and long as necessary to fit the plates in the TLC containers.
2. Draw with a pencil a parallel line at 1.5 cm above the lower border. Divide it with separate marks at intervals of 1 cm and separate it 1 cm from both sides of the plate.
3. Write with a pencil the initials of the sample and patterns below each mark. It is very important to correctly choose the patterns in each plate, because the precision of the analysis is highly linked with the choice of the patterns.

Spotting the samples

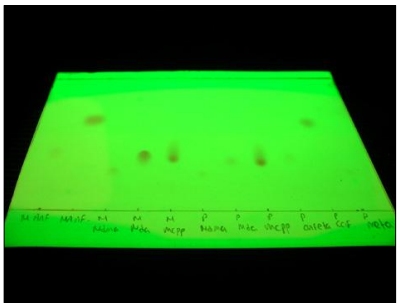


- Drop 3 µL of the sample on the TLC plate using a capillary micropipette.
- Drop the appropriate µL of the pattern with a different capillary micropipette. Different µL of each pattern will be added depending on its concentration.
- Let the solvent evaporate.
- Look at the stains that the samples and patterns have left on the TLC plate using the ultra violet developing camera. If the procedure has been done correctly, and the filling has been sufficient, dark stains should be seen at the spotting site. If they cannot be seen, spot again until the stains are visible.

Elution

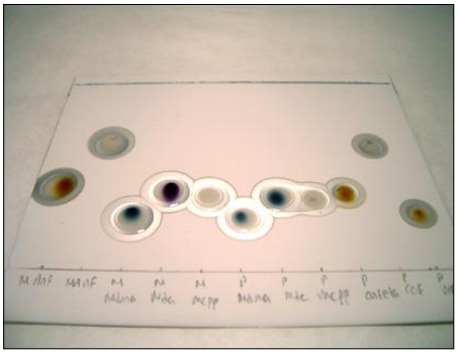
1. Introduce the TLC plate, using tweezers, into the glass container, and let the top of it lean against the dryer paper while the bottom is fixed at the small glass lump of the container.
2. Seal the glass container with its lid.
3. Leave the TLC plate inside the container with the elution until the solvent has gone up until reaching the upper line (previously drawn with pencil onto the TLC plate: See Step 3).
4. Take the TLC plate out of the container and let it dry in a ventilated place.

Visualization UV



- Introduce the dry TLC plate into the ultra violet developing camera.
- Compare the sample stains with the pattern ones.
- Circle the top of the stains with a pencil.

Visualization Reagents (see Annex for more information on colorimetric tests)



1. Add a drop of Marquis Test to the stains. After a few seconds the sample should color depending on the different substances. This process is useful for the identification of MDMA, phenethylamines, amphetamines, cathinones, cannabinoids and other substances.
2. Add onto the developed stain a drop of p-DMAB-TS Test (ethanolic para-dimethylaminobenzaldehyde). After a few seconds the sample should color according to the different substances. This test is useful for the identification of indolamines (LSD, magic mushrooms, foxy, 4-AcO-DiPT, etc) and for some phenethylamines (2C-T-2, 2C-E, DOM, etc.).

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Quantification

Thin layer chromatography can be used to determine the relative concentration of a component when compared to a standard. This is only a comparative semi-quantitative method. Approximate concentrations can be estimated by bracketing the observed sample concentration within appropriate standard dilutions. For more accurate quantification image processing software may be employed.

Ultraviolet spectroscopy is a very useful for quantization. EC uses this method with the following characteristics: solutions of the samples in methanol are passed by a spectrophotometer on 200-400 nm scan wavelength (ultraviolet). The absorbance values are compared with those of standards or by the extinction coefficient calculating the concentration of the sample.

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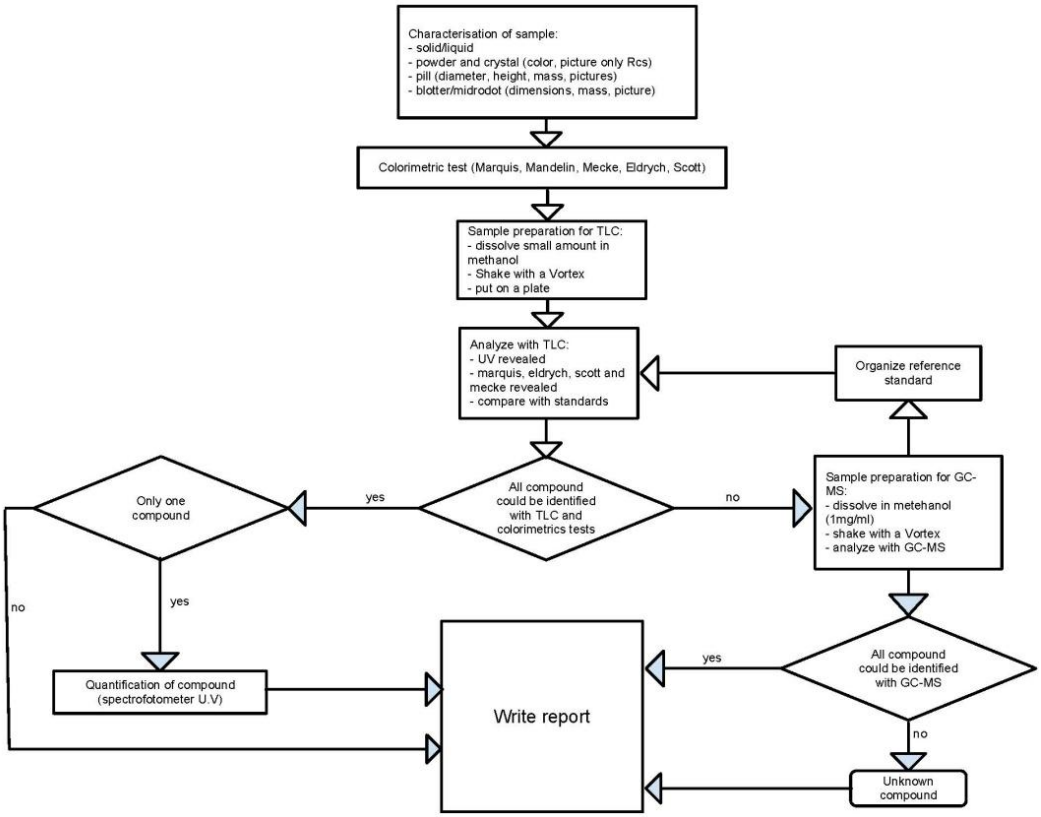
Analysis Method Flow Chart (EC method)



03

Gas Chromatography/Mass Spectrometry (GC/MS)

The description of this method comes from the experience of the Drug and Information Monitoring System (DIMS), located at the Trimbos-Institute in the Netherlands. This procedure is validated according to the principles described in ISO 15189.

The Drug and Information Monitoring System (DIMS) was established in 1992 to prevent serious health problems that could arise as a consequence of the consumption of illicit drugs. To this aim, drugs are chemically analyzed by a sub-contracted laboratory which, until 2009, was the Deltalab Laboratory in Rotterdam. From then on this service has been provided by the DSM Resolve Laboratory in Geleen, The Netherlands.

DSM Resolve analyses a fixed amount of drug specimens with a yearly budget. All extra analyses and associated costs are billed separately. The service contract includes: weekly chemical analyses, reporting back to DIMS, feedback, and all material costs associated with the analyses. The agreement also stipulates that intellectual ownership of the drug data belongs to DIMS and that nothing concerning this data may be reported without prior consultation. The laboratory applies proprietary analytical methods.

Introduction

Gas chromatography (GC) analysis is a widespread methodology employed to confirm the presence of chemical substances. This makes it attractive for drug and drug contaminant testing. GC analysis separates the components of a drug sample and a spectral output is generated.

Basically, the drug sample is inserted into the injection port of the GC instrument. The sample is then vaporized and the various components of the sample are separated and identified using an appropriate detection system. The temperature of the GC injection port must be high enough (typically higher than 250ºC) to vaporize a liquid specimen instantaneously. If the temperature is too low, separation is poor and broad chromatographic peaks, or no peaks at all, are detected. If the temperature is too high, the drug compound may decompose or change its structure, giving rise to no detection of substances or falsely identifying them. Also, the carrier gas is of importance, if the gas (for example helium) reacts with the

substances in the drug sample, broad peaks will result. Typically inert gases are used. The time elapsed between injection and elution is called the "retention time" (RT). RT helps to differentiate between compounds. However, RT is not always a reliable parameter to determine the unique identity of a compound. If two drug samples do not have overlapping RTs those samples are different substances. Identical RTs for two drug samples do not, however, exclude the possibility that they are in fact different substances. Potentially thousands of chemicals have the same RT, peak shape, and detector response. Consequentially, it is essential to couple GC to mass spectrometry (MS), collectively termed GC-MS. MS identifies substances by impacting molecules with high energy electrons, this results in their fragmentation into ions with different masses and electric charge (other forms of ionization are also possible, this one is the most common and known as electronic impact). Ions are accelerated through a magnetic field; differences in mass and electric charge determine their speed and the time to reach the detector. The proportion between ions of different masses for a given compound defines its mass spectrum, which is unique for the compound concerned. The intensity of ions is proportional to the amount of a given compound present in the sample. Therefore, the mass spectrum combined with the RT allows the indisputable qualitative identification of compounds while intensity of fragments of ions at this RT allows its quantitation. Mass spectra observed in a given sample are typically compared with computerized (or printed) libraries of reference for the identification of compounds. Mass spectra for psychoactive substances and other drugs are available to the laboratory through relevant chemical and toxicological libraries, either through contacts with forensic institutes or through international connections with the EMCDDA and the Early Warning System.

Summary of analytical characteristics:

Benefits: combining GC with MS is ideal, making qualitative and quantitative determination possible. Results are highly accurate and small amounts of drugs are needed for analysis. Through the use of an adequate library of mass spectra it is possible to identify the substances in a sample.

Difficulties: the difficulty of the technique is that it is relatively expensive (investment, running costs, and maintenance) and requires specialized laboratory technicians.

The costs of the technique for the association: the contract with the laboratory is for a maximum of 100 samples per week, this includes LC-DAD quantification of 10 frequent substances and GC-MS of all substances. Special requested analyses and quantifications of unknown or rare substances are excluded.
For a quantity of 100 drug samples, the cost is 31 € per sample. The extra requested analyses cost 82 € per sample. This low price for each sample is linked with the high number of samples analyzed for the laboratory. In other conditions, the price would be higher.

Costs of the technique for the laboratory: the costs described above include the cost of a specialized technician to do the analyses and to report the results back to the DIMS bureau. Also, use of equipment is included in these costs.

Turn-around time of the technique: the total runtime of a single specimen may take up to 30 minutes, excluding sample preparation and machine maintenance.
The interpretation of results is usually fast with routine substances (within two hours for 100 drug samples), but this can vary a great deal. Some samples are complex mixtures of substances and could contain various unknown substances (unexpected peaks). This may require additional time for analysis.

Qualitative or quantitative method: both.

Reliability of results: it is a highly reliable and reproducible method, which has been previously well described and documented by many groups (forensic, SAMHSA, NIDA, DEA, etc.). It is the golden standard.

Staff requirements: a laboratory technician specialized in GC and MS technology.

Suitable for on-site: not suitable for onsite testing.

Implementation & organizational recommendations for the technique: the contract with the laboratory is based upon the bulk sum available for testing and analysis each year. This is a regular amount of money that is financed by the Ministry of Health and it guarantees at least 100 analyses each week. Yearly, about 5,200 specimens can be analyzed. In addition, because the DIMS is a regular system that is kept very well up-to-date, a lot of tablets circulating on the streets can be identified. If recognized, these tablets are not usually sent to the laboratory, but information about them does end up in the collective database.

MATERIALS & METHODS

For testing in general: plastic gloves and clean materials are used. In case of uncertainty about the specimen's main constituents, Marquis Reagent test is applied (for more information about colorimetric tests see Anex). About 50 mg of sample is needed by the laboratory and whole tablets or dots, are kept dry in a vial or in a plastic wrapping. Rather than asking for smaller quantities, 50 mg is requested in case of erroneous analysis. The drug samples are sent every Monday to the laboratory, by means of an express delivery service. On Thursday the analysis results are sent back in the form of a database document and are uploaded to the DIMS website. This website is available to all the DIMS network partners, that is to say, the testing facilities of all the institutes for addiction and preventive care in The Netherlands. The professional staff from the institutes communicate the results to the drug users who had provided the samples.

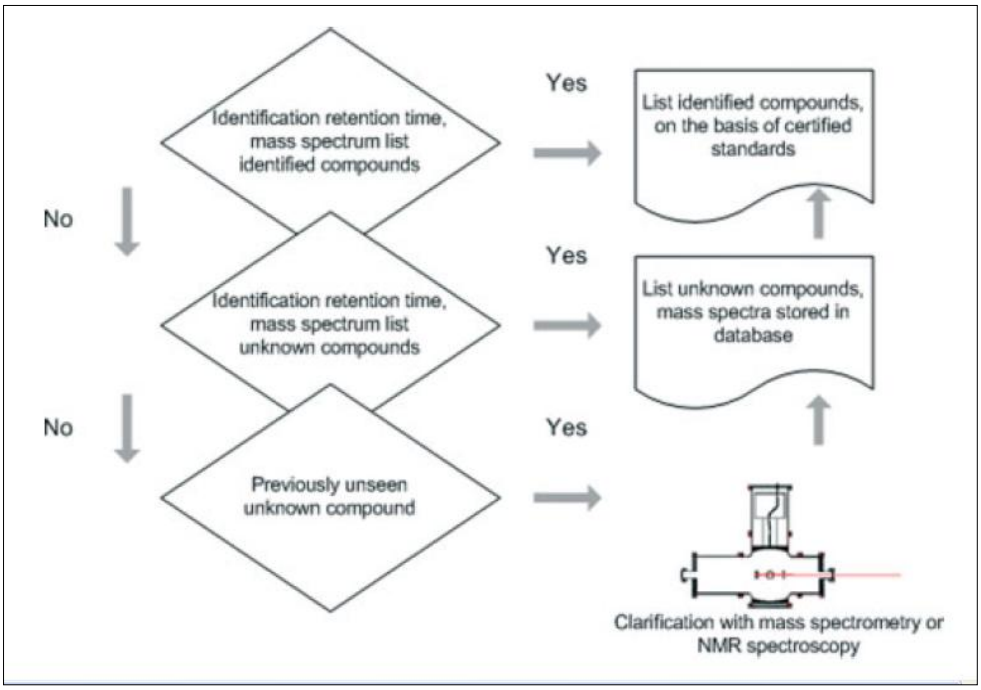
For GC/MS:

Gas chromatography–Mass-spectrometry (Interscience GC8000-Varian Saturn 4D) after LLE (liquid-liquid-extraction): an extraction using Toxitubes®A is performed, using 1 µl from the solvent layer. 1µl of the upper layer was brought, after 5-times dilution in hexane (final volume of 5ml) to a GC-column (WCOT-CP-Sil-8-CB, length 25 m, id 0.32 mm df 0.25 micron) cold on column injection. The initial column temperature has to be low (30-90°C) for high volatility of bases, for example: 30°C, for 0.5 min, then to 280°C, at 12°C/min, hold final temperature for 16 min. The detector temperature is set at 300 °C. For the validation of the GC-MS procedure, an internal standard is regularly injected following the above procedures. This standard is tridecane.
The substances are identified in the full scan mode by electron impact (EI) and confirmed. Mass spectra are compared with the NIST-library and confirmed by comparison with a standard.
The following table shows the retention time of the most analysed drugs and their principal ions detected by the mass spectrometer.

Substance	Retention time	Principal ions (*m/z)*
Caffeine	3.7 min	42, 55, 67, 82, 109, 165, 194
Amphetamine	4.0 min	44, 65, 91, 103, 120
Levamisol	4.3 min	73, 101, 127, 148, 204
Methamphetamine	4.55 min	58, 91, 134
MDMA	4.7 min	42, 51, 58, 77, 135

Ketamine	5.2 min	138, 152, 180, 209
m-CPP	6.3 min	56, 75, 111, 138, 154, 196
2C-B	6.6 min	77, 215, 230, 261
Cocaine	7.0 min	82, 94, 105, 182, 303
Phenacetin	7.3 min	43, 80, 108, 109, 137, 179

Analysis Method Flow Chart:



04

High Performance Liquid Chromatography (HPLC) and HPLC-Mass Spectrometry (LC-MS)

These techniques are presented by describing the Drug Checking services of Checkit! in Vienna, Austria and Saferparty.ch in Zurich, Switzerland.

The Viennese project Checkit! has offered an onsite service since 1997. Checkit! is part of the Suchthilfe Wien gGmbH and is supported and subsidized by the City of Vienna. The chemical analysis is carried out in cooperation with the Department of Toxicology at the Medical University of Vienna.

Saferparty.ch is part of the Youth Counseling Streetwork, a service offered by the Zurich Social Assistance and Welfare Department. Starting in 2001 with onsite Drug Checking, 2006 Streetwork has recently inaugurated the Drug Information Centre DIZ . DIZ is a regular Drug Checking and Consultation service open every Tuesday in the center of the city. Chemical analysis is performed in cooperation with the Pharmaceutical Control Laboratory of the "Kantonsapothekeramt" Bern and RESEACHEM in Burgdorf, Switzerland.

Introduction to the technique

High-performance liquid chromatography (HPLC) is a chromatographic technique which separates mixtures of compounds based on their diverse chemical and physical properties, such as polarity, charge and size, using a solid phase (column) and a liquid phase. It is a method for separation using a suitable detection method (eg. UV absorption or mass spectrometry) for the identification and quantification of substances.

For the identification of synthetic drugs HPLC analysis is a versatile and efficient method. The extent of identification of unknown samples with HPLC alone is, however, strongly dependent on the availability of reference compounds. HPLC methods not coupled to highly specific detection techniques (i.e. mass spectrometry) can yield ambiguous results and are, therefore, often insufficient when complex mixtures of compounds are to be analysed. To enhance the ability to identify substances in complex samples,

HPLC and mass spectrometry methods are coupled. The mass spectra of many substances are sufficiently specific to ensure their identification with a high degree of confidence. A combined technique of liquid chromatography and mass spectrometry, therefore, provides a powerful analytical tool and is the method of choice for screening recreational drug samples.

Summary of analytical characteristics

Benefits: rapid analysis and high resolution. HPLC has a high degree of versatility not found in other chromatographic systems, and it has the ability to easily separate and quantify a wide variety of chemical mixtures. This provides a harm-reduction model for recreational drug users through an extended, differentiated analysis of substances and adulterants in street drugs. It also allows up-to-date pharmacological and toxicological information about the compounds identified.

Difficulties: providing and maintaining a reliable method to analyse street drugs in a constantly changing drug-market. Offering this complex, analytical technique onsite at music events.

Total costs for the technique: start-up 100,000 €

Duration of analysis: between 3 and 20 samples per hour can be analysed, depending on the equipment configuration provided.

Qualitative or quantitative method: both.

Reliability of results: very high.

Staff requirements: HPLC needs 1 or 2 laboratory technicians trained in the analytical procedures and in basic pharmacology/toxicology of drugs.
HPLC-MS needs 1 or 2 laboratory technicians and 1 or 2 university graduates (chemists and toxicologists) trained in the analytical procedures and basic pharmacology/toxicology of drugs.

Suitable for on-site Drug Checking: yes

Implementation & organisational recommendations for the technique: the ability to test onsite requires a well-designed, rugged analytical method that is capable of yielding sufficient data under the prevailing onsite conditions. Such conditions have to be taken into account during the planning process. Furthermore, field testing is necessary to ensure the robustness of any results obtained.

Equipment for HPLC-MS[4]

Material for	**Saferparty.ch** Youth counseling Streetwork, Zurich	**checkit!** Suchthilfe Wien gGmbH
Sample characterization	▲ analytical balance ▲ caliper ▲ digital camera	▲ analytical balances ▲ caliper rule ▲ digital camera ▲ sample vials ▲
Sample preparation	▲ solvent: methanol/water ▲ triethylamine ▲ flasks ▲ ultrasonic ▲ medical syringe with needle ▲ polyamide filter 0.45□m ▲ HPLC vials	▲ solvent: methanol ▲ centrifuge ▲ 100ml of internal standard solution ▲ 400µl Eppendorf vials ▲ vortex mixer ▲ pipettes (10µl, 200µl, 1000µl)
HPLC system	▲ Agilent 1100 series with DAD (approx. CHF 75'000.--) ▲ Column: Macherey-Nagel, Nucleodur C18 HTec 3µm, 250x4.6 (approx. CHF 1'000.--) ▲ Solvents: Phosphate-buffer pH=2.5, acetonitrile ▲ Software: Agilent Chemstation	▲ LC-Packings Autosampler (carousel) ▲ LC-Packings dual gradient Nano-HPLC-system (Ultimate) ▲ Jasco Pump (Model PU-980) ▲ Jasco gradient unit (LG 980-02) ▲ 2 column heaters ▲ Dionex DAD (PDA-100) ▲ Dionex DAD (UVD 340) ▲ Agilent DAD (1100 series) ▲ Columns: 1 Luna PFP 3µ 3x150 mm ▲ 2 Luna PFP 3µ 2x150 mm ▲ Software: Dionex Chromeleon Version 6.80, Chromeleon Commander (administration software) ▲ Solvents: Acetonitrile, Ammonium acetate buffer pH 4.7 ▲ LC-Packings Switchos (2x six-port valves) ▲ Dionex UCI100 Chromatography Interface ▲ Vacuum degasser (Degasys DG-2410)

[4] The prerequisites regarding the detailed analytical equipment can vary from laboratory to laboratory, depending on the setup of the whole analytical system.

HPLC-MS System		• Thermo Surveyor(R) MSQTMPlus • Edwards vacuum pump • Flow splitter from HPLC-System • Nitrogen generator MS-Software: Xcalibur(R)
GC-MS system	• Agilent Technologies 7890A with MSD 5975C (approx. CHF 150'000.--) • Column: HP-5MSI • Software: AMDIS	• analytical balances • caliper rule • digital camera • sample vials •
NMR system (only for new compounds)	• Bruker AVANCE 300	
LC-MS system (only for new compounds)	• Agilent 1100 series with MSD/SL • Column: Macherey-Nagel, Nucleodur C18 HTec 3µm, 250x4.6 • Software: Agilent Chemstation	

Methods (for onsite Drug Checking)

In order to operate the HPLC-System for an event-operation a number of preparatory steps are necessary. In the following, preparation of the HPLC-MS-System in the base laboratory and onsite are both described.

Usually a few milligrams (approximately 5 mg) of the substance to be analysed are needed. Clients requesting a test have to scrape off a small amount of the tablet or the powder themselves. The chemical analysis is based on HPLC-MS (High Performance Liquid Chromatography – Mass Spectrometry). The system can yield qualitative results (identifying substances) as well as quantitative results (substance content in milligrams or milligrams per gram sample). Information on the content of samples is provided to the clients who brought the pills, to the public at large, and also to other organisations in a concise manner.

In order to operate the HPLC-System for an event-operation a number of preparatory steps are necessary. In the following both - preparation of the HPLC-MS-System in the base laboratory and on-site is described.

Preparations for the event when analyses are provided in the base laboratory

As a first step, all solvents (buffers and organic solvent), the internal standard solution, and the calibration solutions needed for analysis are prepared a few days before an event. The calibration solutions contain the substances to be quantified. In order to perform a quantitative and qualitative analysis, the system must be previously calibrated. Consequently, calibration solutions of three different, known concentrations are injected and interpreted with the chromatography software. The acquired chromatograms must be checked and, if necessary, corrected manually. To confirm the success of the calibra-

tion, a control test-sample of known concentration is injected and quantified. The respective sample is prepared according to the prevailing standard operating procedures for onsite-analysis. If the amount in the test sample correlates with the true value, the calibration is valid and the analysis can be started.

Onsite operations

On the day of the event the LC-MS-System, which is mounted on a mobile steel-rack, is transferred to the bus and secured. In addition, all required chemicals, tools, and spare parts for the LC-MS-System are stored in the bus.

At the site of analysis the bus is supplied with electricity in order to power up all systems. After providing power, the workspaces for sample preparation, system-administration and interpretation are installed. Approximately two hours before commencing analysis, the system is started, HPLC pumps are purged, and subsequently the columns equilibrated. A test sample is then injected for a second time to assure the system-stability under the prevalent conditions at the site of analysis. If the obtained amount of the test-sample correlates with the true value, the analysis can begin.

Practical differences between HPLC and HPLC-MS

Operating HPLC alone is, in general, less complicated than HPLC-MS coupling. The reason is that the mass spectrometer needs a sufficient vacuum (provided by a vacuum-forepump), and for most MS applications a constant supply of nitrogen gas from a gas bottle or a nitrogen generator. For HPLC-MS you need the possibility to integrate it into a bus or in another vehicle. HPLC can be used directly at the venue with little preparation needed which makes it easier to employ it for onsite analyses. In addition, HPLC is cheaper than HPLC-MS. HPLC-MS is clearly the more selective method, because it facilitates the identification of unknown substances in drug samples, but a great deal of experience, knowledge, and time is needed for the sufficient interpretation of the acquired results i.e. mass spectra. Whilst HPLC-MS provides a wider range of analytical tools than the HPLC method alone, it does signify spending more time, resources and money to develop an onsite drug checking service.

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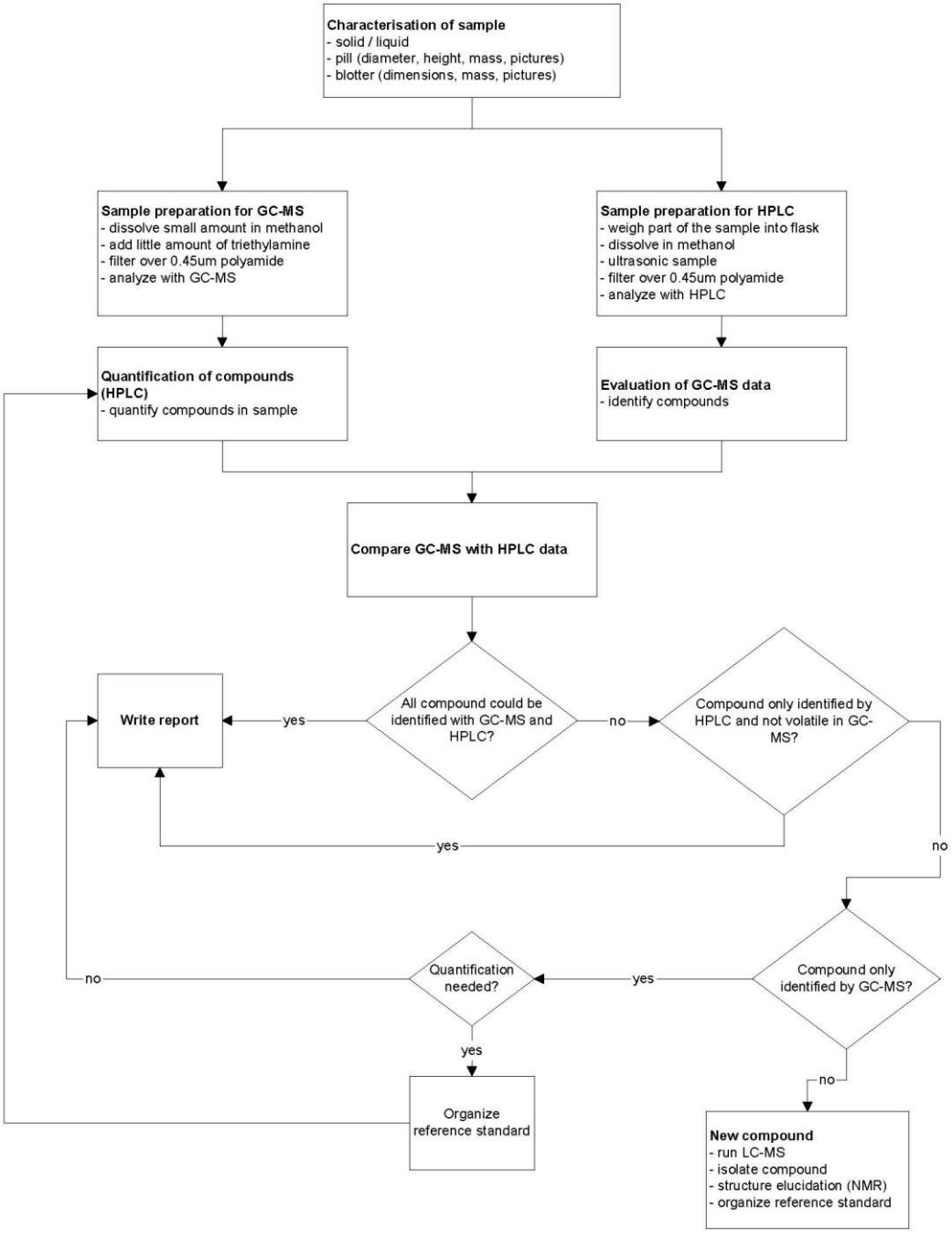
Analytical Method Flow Chart for Saferparty.ch



05

Nuclear magnetic resonance (NMR)

The description of this method comes from the experience of Ailaket, in the Basque Country, Spain.

Since 2002, Ailaket has been working with the General Research Services (RMN-SGIker) of the University of the Basque Country (UPV/EHU).

Introduction to the technique

High field (>500MHz) quantitative NMR spectroscopy (qNMR) for samples in solution state has emerged recently as a valuable tool in drug analysis. Unlike separation techniques, such as high performance liquid chromatography (HPLC) or gas chromatography/MS commonly used in drug analysis, qNMR provides an alternative full molecular identification which is unaffected by the availability of reference compounds (pharmacopeia standards) or the liability of accurate retention time measuring. Modern 2D Fourier Transform (FT) technology permits the analysis of drug samples in non-deuterated aqueous solutions by applying water-suppression pulses (e.g. WATERGATE). This greatly lowers the need for expensive deuterated solvents, eliminates sample preparation drawbacks, shortens the total duration of the analysis to a few minutes, and facilitates the identification of unusual novel drugs or the presence of very polar adulterating substances (such as sugars and amino acids).

On the other hand, the incorporation of water-soluble internal standards (e.g. trimesic acid) or electronic reference pulse signals (e.g. ERETIC) makes it possible to quantitatively compare the integration signals of all the components of the sample at the same time in a single experiment, without the need for accurate sample weighing. In this way, by selecting the appropriate signals for each component, it is possible to establish their relative proportion, molar or weight %, in the sample just entering the data into an MS-Excel spreadsheet. By using the standard autosampling robots, in combination with auto-tuning and auto-shimming gradient protocols, up to 20-30 spectra may be registered per hour. The method, however, is limited to the soluble fraction of the sample. Insoluble components are separated and analyzed apart, if needed, using other techniques.

Summary of analytical characteristics

Benefits: the technique is non-destructive in nature, which means that the sample can be recovered after solvent evaporation, if required.

Each experiment usually provides qualitative and quantitative information of all the compounds of a sample, including drugs, adulterating substances, solvent residues, etc. without the need for reference standards.

The technique is suitable for characterizing novel drugs, synthesis byproducts, unusual adulterating substances, and routine metabolites.

Some components containing 31P (psilocybin, etc…) or 11B (magic, etc…) can also be identified.

Difficulties: the technique is non-portable, it is made up of a 600 Kg magnet, and thus not adaptable for street-work. A limitation of the 1H-NMR method is its inherently low sensitivity when compared to HPLC or GC-MS. However, using high field facilities (>500 MHz) allows proper analysis with samples in the 1-5mg range.

Total costs for the technique:

1. Bruker Avance500 (two-bay) spectrometer (approx. 450,000 €)
2. Two-channel inverse detection probe (BBI 1H/31P-109Ag) with gradients on the Z axis (20,000 €)

Price of the NMR analysis: SGIker provides the following prices to Ailaket! which include solvent, glassware, structure interpretation, and report writing.
60 €/Sample (+ VAT), on the basis of a 300 analyses per year.
70 €/Sample (+ VAT) for single, standard samples.
120 €/Sample (+ VAT) for urgent, unknown samples requiring cumbersome structure elucidation.

Running costs: UPV/EHU is a non-profit organization (public university) and provides analytical support (RMN-SGIker) to its own scientific researchers, to industrial R&D units and to research associations (Red Vasca de Ciencia y Tecnología) at reduced technician staff service prices.
An estimated average per sample cost includes deuterated solvents (4 €), NMR sample tubes (10 €), NMR N2 and He cryogen filling and spectrometer maintenance (15 €), regulated waste disposal (1 €), sample preparation and weighting of solids (10 €) data interpretation and report writing (20 €).

Analysis duration: for standard samples a typical 3 min sample preparation, 2 min experiment registration, and 5 min data analysis is required.
From receipt of an urgent single sample, a report can be delivered by email within 1 day.
A typical batch of 20-25 samples is analyzed within 1 week from receipt.

Qualitative or quantitative method: a typical NMR analysis report provides a listing of all the identified water soluble components of the sample, including their structural formulae and IUPAC names. Optionally, the original 1H-NMR spectra or FID files can be made available for skilled users.
It also provides the w/w % concentration (±1 approx. accuracy) of each component and the percentage of insoluble solids.

Reliability of results: NMR analysis is widely recognized as one of the more reliable techniques for organic structure characterization. Reliability is practically complete for pure or concentrated samples of a given analyte.

For very minor components (< 5%) characterization is still fully reliable, but accurate quantification is more difficult, whereas for trace compounds (< 1%) the reliability depends largely on molecular complexity and is generally poor.

Staff requirements: highly qualified technicians are required to operate NMR facilities. The 500MHz spectrometer of SGIker is operated by a Ph.D. chemist, after a one-year post-doctoral stay abroad, who regularly attends international symposia or meetings to remain aware of recent advances in the area.

Suitable for on-site: high-field NMR spectrometers are basically comprised of an ultrasensitive magnet containing inner liquid nitrogen and helium, high vacuum vessels and a pulse sequence synthesizer. Each component accounts for more than half a ton in weight. Obviously, with the current technology, the system does not allow in-situ street sampling. Samples must be brought to the SGIker center.

Implementation & organizational recommendations for the technique: as this is an expensive technique it is necessary to be selective when using it. After an initial agreement between the Basque Government and the University this is renewed every year and there is an internal contract to carry out at least 300 samples per year.

Materials & methods

Equipment

1. Bruker Avance500 (two-bay) spectrometer (location: Joxe Mari Korta R&D Centre) (approx. 450,000 €)
2. Two-channel inverse detection probe (BBI 1H/31P-109Ag) with gradients on the Z axis (20,000 €)
3. High-precision temperature control unit –150 to +400C
4. BAX-60 programmable Bruker autosampler
5. MNR software: TopSpin 1.3 PL10 (Linux redhat 3)

Sample preparation

6. Deuterated water (10%D) for internal lock (0.6 mL/sample)
7. NMR sample tubes
8. Sample vials
9. Pipettes
10. Analytical balance

Data processing

11. Standard PC or Laptop, preferably under Linux OS
12. MestRec Software (NMR online/offline data processing)
13. MSExcel Software

Methods

Samples (about 5 mg) have to be collected in clean plastic (Eppendorf) or crystal vials (< 2 mL) using NMR or lab spatula and tweezers, avoiding any contact with skin sweat by protecting hands with latex gloves and using a calliper (recommendable).

After proper labelling using organic solvent-proof ink, the samples are stored in 20-30 unit vial holder boxes at 0-5C, and sent by courier (MRW logistic services) to SGIker, together with an identification listing of all compounds.

Upon reception of the NMR analysis results by email, they are published by Ailaket! within 3 weeks.

Waste residues and solvents are processed by a specialized recycling company contracted by UPV/EHU, according to the highest standard protocols available for research chemistry laboratories.

Contact for further information

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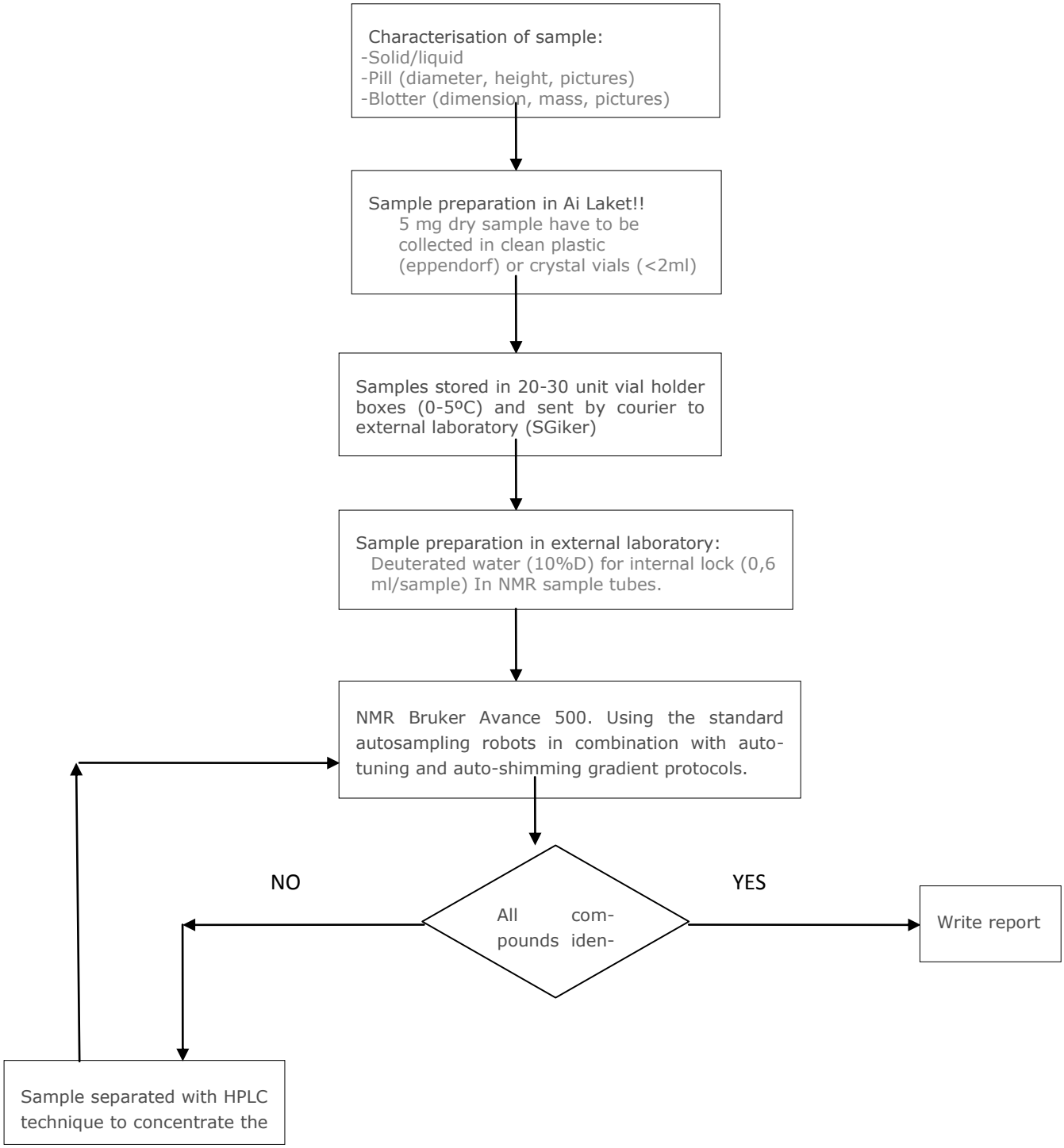
Analytical Procedure Flow Chart



ANNEX: Colorimetric tests

The first analytic technique which is recommended before using any of those described in this guideline is the colorimetric test. This technique consists of applying a few drops of a reactive directly onto the substance to be analyzed. A color is produced as a result of this reaction which permits identification of the substance that has reacted.

The reagents used in the colorimetric tests and the substances they can detect are the following:

- Marquis for MDMA, MDA, amphetamine, methanphetamine, methylone, butylone, MDPV, Bromodragonfly, 6-APB, phenethylamines generally, MPA (methiopropamine), opiates, and synthetic cannabinoids type aminoalquilindole o naphthoylindole (JWH-, AM-, WIN-, etc.).
- Mecke for MDMA, MDA, DXM and methoxetamine.
- Mandelin for Ketamine, amphetamine and MDMA.
- Eldrich (p-DMAB-TS) for indolamines (LSD, DMT, foxy, 4-Aco-DMT, etc.) and for some phenethylamines (2-CTX, 2CE, DOM, etc.).
- Scott for cocaine and mephedrone.
- Water solution of $FeCl_3$ for GHB.

The table shows the colors for each reactive and substance:

SUBSTANCE	MECKE	MARQUIS	MANDELIN	p-DMAB-TS	SCOTT	FeCl3 solution
MDMA, MDA						
Amphetamine						
Methamphetamine						
2C-B, DOB						
2C-I, DOI						
2C-C, 2C-D, 2C-E, 2C-TX, 2C-P, DOM, DOC						
Bromo-Dragon-Fly						
Mescaline						
6-APB						
Phenethylamines	various colors	Various colors				
Methylone, Butylone						
MDPV						
Mephedrone						
LSD						
DMT, 5-Meo-DMT, DIPT, 5-Meo-DIPT, AMT						
4-Aco-DMT, 4-Aco-DIPT, 4-Ho-MET						
Tryptamines				Various colors		
Cocaine						
Opiates (heroine, morphine, codeine, etc.)						
Ketamine						
Methoxetamine						
Synthetic cannabinoid (JWH-, AM-, WIN-, etc.)						
GHB						
MPA						

To ensure operator safety in the use of these reagents latex or vinile gloves, protective eyeglasses and clothing should be employed. In the case of acid reagents (marquis, mecke, mandelin and eldritch) a solution of sodium bicarbonate or some mild base to neutralize eventual spills and splashes should be on hand.