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Identification of Organic Substances such as New Psychoactive Substances or Designer Drugs using FT-IR Spectroscopy

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The table shows the most important changes that has been done compared to the latest former version.	
Date of the latest former version:	2018 initial version
Section	**Changes**
All sections	Layout harmonisation according to new template and minor linguistic changes
1.1.	Clarified field of application
1.2	Clarified limitations of this method
3	Added reminder of safety instructions
7.	New section

Identification of Organic Substances such as New Psychoactive Substances or Designer Drugs using FT-IR Spectroscopy

1. Scope

1.1. Introduction and field of application

The identification of the chemical structure of an unknown substance is required for purposes of control by enforcement authorities whether for verification of correct classification by customs or detection of an illicit substance. This method is especially applicable for pure substances since the methodology lack a separation step. Depending on the instrument, the procedure may also be applied for samples containing up to three components, if a software-based subtraction of spectra can be carried out.

The method can be used to identify substances such as narcotics, drugs, new psychoactive substances, chemicals and pesticides,using appropriate reference libraries for these categories of compounds.

The method is also suitable for recording a spectrum that can be added to a reference library once a new substance is identified.

This document is a general description of a method applicable and suitable for the scope presented in 1.2. It is reminded that national legislation(s) may require more stringent specific requirements for detection of narcotics for analytical results to be used in a criminal justice context.

1.2. Scope

Qualitative analysis of pure compounds or preparations by FT-IR spectroscopy. This document presents the minimum requirements for this analysis procedure.

The identification is very reliable for pure substances and most isomers can be clearly distinguished. The amount of a substance should be more than approximately 5 to 10 % to be detected. Optical isomers, e.g. dexamphetamine and levamphetamine cannot be separated. For preparations that are more complex and preparations at low concentrations, e.g. proteins on mannitol, the procedure is not appropriate. The method is not suitable e.g. for small quantities of heroin in mixtures of acetaminophen and/or caffeine or mixtures containing cocaine and boric acid or preparations of amphetamine with sugar and caffeine.

If the substance is not pure or if all known isomers are not available in the reference library, this method should be used with care.

2. Principle

FT-IR stands for Fourier Transform Infra-Red, the preferred method of IR spectroscopy. In IR spectroscopy, IR radiation is passed through a sample. Some of the IR radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. A substance is identified by comparing the spectrum to a reference spectrum. Like a fingerprint no two unique molecular structures produce the same IR spectrum and e.g. regioisomers (molecules with the same parent structure with the same functional group in for example para-, orto-, or meta-position) can be distinguished from each other. This makes IR spectroscopy useful for several types of analysis.

3. Reagents and materials

3.1. Ethanol (p.a.)

3.2. Caustic soda 5% (p.a.)

3.3. Hydrochloric acid 5 % (p.a.)

3.4. Suitable volatile organic solvent (p.a.), e.g. diethyl ether, heptane, methylene chloride, methanol, ethyl acetate

3.5. Magnesium sulfate, anhydrous

All above reagents should be handled and stored according to their safety instructions.

4. Apparatus

4.1. Recommended instrument parameters:
- wavelength resolution: 4 cm-1
- range: 650–4000 cm-1
- at least 16 scans per spectrum.

4.2. ATR-module (Diamond crystal recommended)
4.3. Computer with software
4.4. Tools for sample preparation or grinding
4.5. Spatula
4.6. Pipette
4.7. Glass vial with screw cap (2 ml)
4.8 NaCl plate

5. Procedure

5.1. Sample preparation

5.1.1. Solids

Solids and powders are crushed if necessary and a few granules are put on the diamond, so that this is covered. In some cases, grinding of the substance gives a better result.

5.1.2. Liquids

Liquid substances, oils and pastes can be used directly by covering the diamond with a small drop. Slightly volatile substances may be covered for example with a NaCl plate.

5.1.3. Crystallised substances

The infrared spectrum of crystals can be influenced by its crystal structure and may differ from the spectrum of the library. Therefore, closely related substances (e.g. some designer drugs) should also be measured as a neat film.

To generate a neat film, 1-2 mg of the substance is dissolved in 0,5 - 1 ml diethyl ether or other suitable volatile organic solvent (e.g. methylene chloride, methanol) in a small vial. One drop is transferred with a glass pipette directly on the ATR crystal where the remaining solvent is continuously blown off until dryness.

5.1.4. Salts

Free bases can be obtained from their salts (e.g. hydrochloride) by alkaline extraction.
For generating the free base, 2 - 5 mg of the salt (e.g. hydrochloride salt) is dissolved in 1 mL demineralized water and alkalized with one drop of NaOH (5 % w/w). The solution is extracted with 1 mL diethyl ether or other suitable volatile organic solvent (e.g. methylene chloride, ethyl acetate, heptane).

The organic phase is transferred in a new vial and the solvent is evaporated under a gentle nitrogen flow until the volume reached approximately 500 µL.
The remaining fluid is transferred with a glass pipette directly on the ATR crystal where the remaining solvent is continuously blown off until dryness.
Alternatively, the solvent can be completely evaporated at room temperature and the remaining material recorded, as such. It is recommended to treat the extract with a little amount of dried magnesium sulfate (or similar drying agent). The drying reagent should be removed before evaporation of the solvent. All solvents and reagents used should be of analytical grade

5.2. Measurement of the blank/Background

Prior to the measurement of a sample, a so-called background spectrum needs to be collected.

5.3. Results and spectroscopic proof

The spectrum should be of good quality. The spectrum should show sufficiently intensive absorptions and should optimally contain no negative absorptions or baseline drifts. Baseline correction, smoothing or normalization should only be carried out in exceptional circumstances.

A library search generates a match number often referred to as a hit quality index (HQI). A good match score is more than 80 out of 100 for a pure compound. One should keep in mind that spectra of free bases and salts of the same compound can differ, resulting in lower match ratio's if the library is not equipped with every salt and/or free base of the target compound.

For a positive identification of a substance, the spectrum should show all absorptions of the library spectrum and no additional absorptions.

The identity of a substance could be verified by more than one analytical method (e.g. GC-MS, NMR) to make sure that there is no confusion between similar substances. Then, the corresponding spectrum can eventually be added in the user library of the instrument. Eventually, it can be also considered for integration in a shared public library or database (practical considerations regarding this process are presented in Annex).

5.4. Libraries

Ideally, a comprehensive collection FT-IR spectra of drugs, new psychoactive substances, drug precursors, cutting agents, and common solvents and chemicals should be present in the library. In practice the libraries installed on the FT-IR instrumentation are based on the proprietary format of the manufacturer of the instument. The libraries provided by the manufacturer, can generally be extended with additional FT-IR spectra available from commercial sources, from public libraries established on the initiative of communities of scientific networks, and by spectra recorded by the laboratory with its own samples.

Spectra recorded on FT-IR instruments from different manufacturers (or also different generation of instruments) can differ in spectral ranges and resolution. Check of similaririty (e.g. through overlay of two spectra) by visual examination by the spectroscopists will still be possible. However search algorithms implemented in the software of the PC of the instrument may not handle heterogeneity in data format and may restrict the search in the data in library for a specific spectral range. The analyst can eventually export its data and use commercial or open source spectroscopic data processing software to overcome such limitations (see Annex).

5.5. Performance check

Periodically, the instrumental performance needs to be validated. These tests include x-axis accuracy, y-axis accuracy, contamination and signal-to-noise ratio. Most instrument will indicate when a verification test is needed.

6. Calculation

Not relevant.

7. Expression of the results

The common name(s) of the identified substance(s) can be used to report the result.

For less common substances and especially NPS It is recommended to also report the systematic IUPAC name(s) of the substance(s) for unambiguous description of the organic species identified. Eventually the chemical structure(s) (developed formula) can also be presented.

8. Precision

Not relevant.

Annex

This annex merely presents basic practical information, recommendations and guidance for enhancing the exploitation of FT-IR data through extended libraries and data processing software.

1 Adding spectra to the user library

It is common practive in most laboratories to add in user library the spectra of compound of interest that can be encountered in the routine aanalytical activity of the laboratory.
When possible it is recommended to ensure a minimum of good practice regarding traceability of the data, for instance considering only data from samples which chemical identity have been confirmed and/or fully characterised with other analytical methods (GC-MS, HR-MS, NMR). The data of chemical susbtances purchased by a chemical supplier can also be considered for enrichment of user libraries.

2 Libraries of interest

Several commercial librairies containing infrared spectra of numerous chemicals are available.
They may already be provided by the manufacturer of the instrument, or alternatively from them or from data suppliers.
Among these, the libraries and databases of list of suppliers given as examples below cover a broad range of products (polymers, food additives, pesticides, insecticides., pharmaceuticals, etc...) of interest for the control of legal trade of chemical susbtances:

-The KnowItAll IR Spectral Database Collection from Wiley:
https://sciencesolutions.wiley.com/solutions/technique/ir/knowitall-ir-collection/

-The Thermo Scientific™ Commercial Materials FT-IR Spectral Library:
https://www.thermofisher.com/order/catalog/product/834-010101

-The S.T. Japan ATR-FTIR Spectra Databases:
https://www.stjapan.de/spectra-databases/atr-ftir-spectra-databases/

The above mentioned libraries and databases may also contain some data of compounds of interest in customs and forensic laboratories. However, more comprehensive and up to date sources of spectra of designer drugs and new psychoactive substances can be obtained from the community and organisations active in the identification and monitoring of such substance, for instance SWDRUGS, ENFSI and the EMCDDA.

The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) works to respond to the needs of the forensic community in particular by monographs and analytical libraries available on its web site:
https://swgdrug.org/

The European Network of Forensic Science Institutes (ENFSI) is also compiling analytical libraries on illicit drugs, and related compounds (eg. cutting agents) and new psychoactive substances. ENFSI libraries are available for the members of the ENFSI network, or on request to the ENFSI Drug Working Group committee.

The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), generally receives the analytical data obtained for the first identification or detection of new susbtances. Laboratories authorized with an official access to the EMCDDA EDND2 database can eventually download the electronic files of single FT-IR spectrum of certain compounds to complete the data in their own user libraries.

3 Search in libraries

As discussed in §5.4, some limitations could be encountered when sharing or using FT-IR data from other sources.
The software of certain manufacturers (e.g OMNIC from ThermoFisher) may allow to partly overcome such limitations, through their options for constituting libraries by reading, and converting data from different proprietary or open format such as:

-IUPAC JCAMP format: https://iupac.org/what-we-do/digital-standards/jcamp-dx/
-SPC binary files, the Thermo Galactic format for spectroscopic data
-CSV files (Text files)

Nevertheless, the various spectral ranges that could be encountered depending of the instrumental original source may remain a limitation for the constitution of libraries that are installed on the computers of the analytical instruments in use in the labs,

This can be overcome by the use of spectroscopic software allowing more flexibility for data processing and search in databases.
One can therefore envisage the use of commercial software such as Spectrus Processor from ACD/labs, Mnova from Mestrelab Research or KnowItAll from Wiley, or alternatives such as Spectragryph-software (https://www.effemm2.de/spectragryph/) or Essential FTIR® Spectroscopy Software Toolbox (https://www.essentialftir.com/).