Customs Laboratories
European Network

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Identification of unknown organic substances such as drugs, narcotics and New Psychoactive Substances by GC-MS

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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
Section	**Changes**
All sections	Layout harmonization according to new template and minor linguistic changes
1.1.	Clarified field of application
1.2.	Clarified scope of the method
5.1	Revised the correct sample preparation depending on matrix
5.2.	Clarified description of tests to control the performance of the GC/MS-system and quality of mass spectra
5.3	Added recommendations for evaluation of peak purity and saturation of MS detector
5.4	introduced a list of available libraries
7.	Extended the instructions and recommendation for reporting substances identified through match in libraries, or for communication of hypotheses of possible chemical structures.
Annex	Selection of relevant literature and web page references

Identification of unknown organic substances such as drugs, narcotics and New Psychoactive Substances by GC-MS

1. Scope and field of application

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, detection of an illicit substance, or reporting of a new designer drug to the competent national and international authorities.

Mass spectrometry is a powerful and flexible analytical technique that provides key information facilitating the determination of the chemical structure of organic substances. However, it has to be considered, that it is, in most cases, not possible to distinguish between regio-isomers of a certain compound. The confirmation of the structure of an unknown compound may therefore require further investigations with an independently second method (i.e. IR-spectroscopy, NMR-spectroscopy).

The present CLEN method is based on the electron impact (EI) ionization technique which is currently the most commonly used for detecting organic substances in GC-MS. The use of chemical ionization in the negative or positive mode (NCI or PCI) may provide additional information for the elucidation of chemical structures in the analysed sample. However, this is out of the scope of the method described in the present document and the analyst should then consult the relevant documentation regarding such applications in the scientific literature and in the instructions of the instrumentation used for GC-MS analysis.

1.2. Scope

This method is a general description of an analytical procedure by GC-MS that can be used to identify substances such as narcotics, drugs, new psychoactive substances, but also chemicals and pesticides, using appropriate reference libraries for these categories of organic compounds.

The method is meant to obtain a spectrum that can be added to a reference library once a new substance has been identified, as it includes minimum requirements for obtaining a result of sufficient quality, to be shared within CLEN community

It is however 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.

2. Principle

Qualitative analysis of pure compounds or preparations by gas chromatography coupled with a mass-selective detector. In the case of a new compound, which is not implemented in the spectral library, certain structural elements can be inferred by characteristic mass fragments. These pieces of information used in combination with those obtained from the results obtained on the sample under investigation with other analytical methods can then be used to formulate hypotheses for structure of the unknown compound.

3. Reagents and materials

3.1. Solvents: ethanol, methanol, chloroform p.a. (other possible solvents that can be used should be of analytical grade)

3.2. Ammonia (to adjust pH when required)

3.3. Capillary GC-column, suitable for use in GC-MS (low column bleeding)
e.g. HP5 – MS, 30 m, 0.25 mm (Diameter), 0.25 µm Film (or equivalent)

3.4. GC vials, with crimp or screw caps/tops

3.5. Common laboratory equipment and materials such as micropipettes, syringes for GC autosampler, 0.45 µm filters etc.

4. Apparatus

4.1. GC/MS-system consisting of:

4.1.1. Gas-chromatograph

temperature programmable oven
inlet-system (split/splitless) (other inlet systems such as PTV may be used)
carrier gas: helium, nitrogen or hydrogen
heated transfer-line to MSD

4.1.2. Autosampler

4.1.3. Mass Selective Detector (MSD) using electron impact ionization technique (EI) (with a quadrupole or an ionic trap afterwards)

4.2. Analytical balance (precision 0.1 mg)

4.3. Ultrasonic bath and centrifuge for preparation of samples

5. Procedure

The unknown organic substance or preparation is dissolved in an appropriate solvent for performing gas-chromatography. Due to its abilities to dissolve a wide range of new psychoactive compounds methanol or ethanol are routinely used as solvents. Examples of alternative solvents are chloroform or acetone.

When using ethanol or methanol, the analyst should keep in mind the possibility of esterification of organic acids present in the sample. This can be easily detected through successive analysis with both solvents and the mass shift of $\Delta m/z = 14$ that can be observed in the corresponding MS spectra arising from the mass difference of the esters formed with these two solvents.

Many of these new psychoactive compounds are amines and are used in salt forms (mainly as hydrochlorides). These salts can be transformed under basic conditions into their free bases to improve peak shape and sensitivity in gas chromatography.

For further identification or to improve volatility a derivatization procedure can be used as presented in annex. For the time being MS libraries based on derivatization are not yet widely shared among enforcement laboratories, therefore such approaches, are not included and fully described in the present method.

If there is a negative or inconclusive result, another solvent could be used, or the concentration of the solution could be increased (e.g. 5 mg/ml or more), but overloading the column should be avoided. In some cases, it is appropriate to convert a sample to its base form or, alternatively, derivatization could be carried out (see an example of procedure in annex).

The samples to be analysed may be present as a solid (powder), tablet, capsule, blotter, plant material, solution or suspension.

Tablets are previously crushed, capsules are opened and an aliquot of the content is analysed.

5.1. Sample preparation and extraction

Each sample should be prepared in order not to saturate the detector (see point 5.3), to reach a sufficient signal to noise ratio and to get a good chromatographic separation.

For powders : About 20 – 50 mg of the analytical sample is dissolved in 2 ml in a suitable solvent.

For crystals (hypothetically pure substance): 5 mg of the analytical sample is dissolved in 10 ml of solvent.

For tablets, capsules and blotters: 1-5 units are dissolved in 2 ml in a suitable solvent.

Any suspension of particles should be eliminated by filtration or centrifugation. If there is a solubility problem, 15 min sonication can be performed before filtering the sample.

For some substances like ephedrine and some ring substituted amphetamines, the detection in the GC MS is not so good when the sample is directly dissolved in suitable solvents. It is better first to dissolve the sample in dilute ammonia (ammonia-water 1:1) and then perform a liquid-liquid extraction with chloroform.

For materials impregnated with NPS:

A **quick** (1-2 min) extraction of 50 mg - 100 mg of the analytical sample is performed generally in Methanol, avoiding to dissolve constituents of the matrix. If needed, the solution is filtered directly into a GC vial through a 0.45 µm syringe filter.

For liquids:

Aqueous solutions, syrups:

- First measure the pH.
- If the pH is more than 5, for direct injection in GC-MS, dilute the sample 1:10 with ethanol or methanol in order to avoid damage of the column due to water.
- If the pH is acid, 1ml of the sample is treated with 1ml Ammonia to reach pH 5-9 and then a liquid-liquid extraction with Chloroform by vortex mixer is performed. The Chloroform extract is subjected to analyses.

  For aqueous or hydro-alcoholic solutions, a drying procedure with Na_2SO_4 can be applied to remove the water before injection. Knowing the amount of water to be removed from an aliquot of sample, one can calculate the amount of Na_2SO_4 needed (one molecule of Na_2SO_4 for 10 molecules of H_2O) and use an excess (e.g. 10%) put together in a vial with the sample for 30 minutes. The supernatant is then injected for the GC-MS analysis.

5.2. GC-MS-analysis

GC recommended criteria:

Chromatographic parameters and column should be chosen in order to obtain the best possible separation, selectivity and sensitivity with optimized time run. The analytical conditions can be established following examples and indications that can be found in many scientific articles and guide books on chromatography.

Mass Selective Detector :

For a **single quadrupole** spectrometer use these general settings (first screening of unknown substance, to be optimized depending on results)

electron impact voltage: 70 eV (mandatory as the spectra is intended for enriching a common reference library)

MSD: Scan range 40-600 amu

MSD Source at 230°C

Solvent delay: 0.5 min after solvent peak (generally 3 min)

Quality control:

The laboratory should have in place a quality control procedure based on the use of some reference compounds or mix that are analyzed from time to time for checking both the performance of the chromatographic separation and the quality of the obtained mass spectra.

This can be achieved by a combination of:

- **checking the mass selective detector**

The check includes the regular setting and checking of the device parameters ("tuning"): the regular review and, if necessary calibration of the mass axis, producing the required mass spectrometric resolution and control of the mass spectrometric sensitivity. This should be done by an autotune or tune evaluation procedure regularly at least every 3 months. For this purpose PFTBA (mass fragments: 69, 219, 502) is commonly used. Modern GC-MS instruments that are used to operate the present method are equipped with software that runs this autotune procedure carrying out tests relative to shift against expected mass of the mass fragments of PFTBA, as well the relative intensities of isotopic peaks or of characteristic fragments expected for the test substance. The analyst should then refer to instructions provided by the manufacturer of to perform the autotune procedure with to procedure implemented on the instrument.

In addition, the MSD must be calibrated after every significant modification to the system (e.g. column switching or switching off the MSD for maintenance). Calibration reports are recorded, for instance in the instrument logbook, for quality and traceability purposes.

- **checking separation, selectivity and sensitivity :**

A mixture of substances with different chromatographic properties should be used. With the help of a known and suitable test mixture the chromatographic separation performance and the sensitivity of the mass spectrometer can be checked simultaneously. The results of the measurement of the test mixture must meet established criteria (e.g. constancy of retention time, distance between peaks (selectivity), peak area/peak height (sensitivity)).

- **checking the absence of carry-over**

The check for carry over can be performed by examination of GC-MS analysis of blank samples.

5.3. Examination of the MS spectra – quality criteria

Peak purity and saturation of MS detector:

Mass-spectrometric detection of a substance should refer to the mass spectrum of a uniform species (peak purity).

Checking the consistency of the mass spectra at various scans of the chromatographic peak allows the detection of possible coelution of various substances, as well as possible saturation of the mass detector. The mass spectra observed for both the front and the tail of the chromatographic peak (e.g. the first three or four scans above the background TIC signal) can be compared to that obtained at the maximum intensity of the peak.

When the front and tail scans show significant differences, a coelution of several substances can be suspected.

When the front and tail scans are very similar but show significant differences with the mass spectra of scans at the maximum intensity of the chromatographic peak, then the analytical conditions were probably incorrect leading to a problem of saturation.

In this latter case a dilution of the injected sample should be performed.

On the opposite, in case of weak signals a higher concentration of sample is advisable when possible.

The elimination or attenuation of instrumental noise and chemical background, arising for instance from column bleeding, can be considered to keep only the most meaningful information of a mass spectrum for further processing and search in libraries. To this aim, the analyst can examine the pattern of signals of the scans before the chromatographic peak of interest. A simple way for determining a cut-off value and the start of signals corresponding to the eluted substance in this chromatographic peak is to identify the main base peak BP1 (and eventually the second base and third base peaks BP2 and BP3) observed for the scans of the chromatographic peaks (e.g. at the top intensity of the peak). This can be achieved when checking the absence of saturation and evaluating the purity of the peak.

The noise can then be estimated over an area, free of chromatographic peaks, up to a retention time as close as possible to that of the cut-off point determined with the apparition of the base peaks observed in the chromatographic peak of interest. The mass spectra of the scans before this cut-off point should not show the presence of these base peaks above the background noise. The processing software can then compute an average background over a number of scans from such an area free of chromatographic peaks before this cut-off point. This background is then subtracted to the mass spectra collected over the whole chromatographic run and especially for the substance under study eluting after this cut-off point. The resulting mass spectra should then show much fewer small signals, distributed all over the range of m/z values, typical of the noise. The resulting spectrum should appear as a finite list of a few major peaks number and a total number of m/z fragments peaks generally well below hundred (at least for the higher m/z values around the base peaks) which can then be used for search for match in libraries. It should be noted that the software of certain instruments already performs these operations of background subtraction prior to further processing and search in libraries. Averaging over a number of consecutive scans of the mass spectra collected in a chromatographic peak could also be performed. Modern software usually keeps track of these operations in the meta-data of the electronic file produced after these processing. The analyst should carefully evaluate and balance the improvement achieved versus possible alterations of meaningful information through these processing operations.

These operations should also be carefully considered when the data are to be used for enrichment of libraries with mass spectra of new substances identified through GC-MS, and other analytical techniques. In this case, it is also useful to support the interpretation with interpretation of the main fragments that are observed in agreement with the rules of fragmentation commonly established in the knowledge of mass spectrometry.

These aspects can be consulted in other documents (e.g. the NIST MS tools manuals and documentation) and are discussed in a CLEN guide document for use of these NIST MS tools software.

5.4. Substance Library search

The mass spectra pre-processed as presented in the previous § 5.3 can then be used for search in mass spectral libraries.
The libraries can be those established in-house, on the same or on another instrument of the laboratory, using known substances (purchased chemical standards or samples fully identified through other analysis such as NMR).
Several libraries, publicly available, can be downloaded as for example:
- SWGDRUG
- Caymanchemical

The National Institute of Standards and Technology (NIST) develops mass spectral libraries and provides related software tools.
Part of the NIST libraries are available as demo (extended libraries can be purchased through distributors)

Other libraries are also available commercially:
- WILEY – Mass Spectra of Designer Drugs

The CLEN MS library is available for European Customs laboratories. It is established with analytical data collected on substances identified and characterised by the Customs Laboratories European Network.

The algorithms used for search in a library are well described in the literature cited in annex. For instance, the basic principles behind the match factors generally computed by the search algorithms are presented in the p 41 of the manual “NIST Mass Spectral Search Program (Version 2.3)”.
These well-established algorithms are generally implemented in the data processing software of the computer of the instrument or of the data processing work station.
While these tools are robust, the analyst should always keep a critical view on the results and not always blindly accept a match even with very satisfactory matching factor.
For instance, a missing fragment in the spectrum under investigation with respect to that found in the reference library should alert the analyst that the hypothesis of chemical structure could be a similar but not identical structure to that found in the reference library.

6. Calculation

Not relevant.

7. Expression of the results

When satisfactory matches are found in libraries for the mass spectra, the common name(s) of the identified substance(s) can be used to report the result. In this case, it is also recommended to mention the libraries used for the search an make sure the common names are correctly spelt and in agreement with the name given in international reference lists corresponding to the category of the found substances. For instance, EMCDDA and UNODC references can be used for New Psychoactive Substances, and international non-proprietary name (INN) for pharmaceutical drugs or active ingredient(s). As a unique identification code, the CUS number can also be added for substance(s) registered in the European Customs Inventory of Chemical Substances (ECICS).

For less common substances not found in validated libraries, the GC-MS results are likely not sufficient for a definitive identification of the substance. Nevertheless, the interpretation of the MS fragments could help the suggestion of possible approaching chemical structures for the analytes. The systematic IUPAC names, as well as chemical identifiers and/or a chemical table file (e.g. SMILES, InChI and InChIKey, Molfiles in MDL or SDF formats) can be used for unambiguous description of these hypothetic chemical structures which can then be further examined when interpreting the results obtained with other analytical techniques (i.e. NMR, HR-MS). Alternatively, the drawings for these hypotheses of chemical structure(s) (developed formula) can also be presented.

8. Precision

Not relevant.

Annex

Literature

- Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites, Part 1-3 - Pfleger, K., Maurer, H.H., Weber, A. ISBN: 978-3-527-34287-7 December 2016.
- Fundamentals of Integrated GC-MS. Chromatographic Science Series, Vol. 7 - Gudzinowicz B. J.,Gudzinowicz M. J und. Martin H. F. Marcel Dekker, Inc., New York—Basel 1976.
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- "The Critical Evaluation of a Comprehensive Mass Spectral Library" P. Ausloos, C.L. Clifton, S.G. Lias, A.I. Mikaya, S.E. Stein, D.V. Tchekhovskoi, O.D. Sparkman, V. Zaikin, Damo Zhu, J. Am. Soc. Mass Spectrom., 1999, 10, 287-299.
- "An Integrated Method for Spectrum Extraction and Compound Identification from Gas Chromatography/Mass Spectrometry Data" S.E. Stein, J. Am. Soc. Mass Spectrom., 1999, 10, 770-781.

Relevant links/sites

- CAYMAN : https://www.caymanchem.com/
- SWDRUG : https://www.swgdrug.org/
- NIST : https://www.nist.gov/srd/nist-standard-reference-database-1a-v17
- WILEY: https://sciencesolutions.wiley.com/solutions/technique/gc-ms/mass-spectra-of-designer-drugs/
- Chemical Identifiers: https://en.wikipedia.org/wiki/International_Chemical_Identifier
- Chemical Table File: https://en.wikipedia.org/wiki/Chemical_table_file