

Establishing Substance Identity by Analytical Characterization

INTRODUCTION

Substance sameness checking is a crucial SIEF activity undertaken during the early stages of the registration process. Establishing substance identity is an important precursor to sameness checking and ECHA is monitoring the quality and robustness of analytical data acquired by registrants to characterize their substances. During the first 5 months of 2009, around 450 enquiries were received, 23% of which were rejected on the grounds that the dossiers were incomplete (e.g. missing spectral data) or the substance identity had not been sufficiently described.¹

In order to demonstrate the identity of a substance unequivocally, it is necessary to characterize it fully using a combination of complementary analytical tests that are appropriate to the type of material in question. It is advisable for SIEF members to agree from the outset on the techniques and methods that will be used to characterize their substance.

In the ECHA Guidance Document for Identification and Naming of Substances under REACH, registrants are advised to collect sufficient spectral data to confirm the structure of their substance and to use suitable chromatographic methods to verify composition and purity. Where there are other main substance identifiers such as morphology, these should also be included.

The table below contains an overview of the various analytical methods that are available for substance characterization. The nature of each substance will dictate which techniques are appropriate: some are suitable for both organic and inorganic materials whilst others are not. Some use solid or liquid samples whereas others involve dissolution. In some instances, e.g. UVCBs, analytical data alone may be insufficient and extra background information such as raw material sources and manufacturing methods should be included. Advice and guidance as to the most appropriate forms of analysis can be provided if required.

Registrants must provide original spectra (paper plus electronic copy as a PDF file if available) or a good quality photocopy, together with sufficient details of their analytical techniques and methods to allow these to be reproduced. Supporting bibliographic references may also be attached if appropriate. Guidance is provided at the end of this document to indicate the type of information required to document analytical data.

¹ ECHA; 2nd Meeting of the Competent Authorities for REACH and CLP (CARACAL), 15-16th June 2009 (Ref CA/57/2009)

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SUITABLE ANALYTICAL METHODS FOR SUBSTANCE CHARACTERIZATION

GENERAL TEST CATEGORY	TYPE OF ANALYSIS	EXAMPLES OF SPECIFIC METHODS	GENERAL REMARKS
Elemental composition	Elemental analysis	Combustion techniques, ICP-MS, ICP-OES AAS, SEM-EDX, XRF	Suitable for organic and inorganic substances. Many different methods available. Can provide qualitative, quantitative and semi-quantitative results. For quantitative results to be meaningful, need to remove solvent and understand purity profile. Useful for providing elemental profile and certain techniques particularly suitable for inorganic solids which may otherwise be difficult to characterize.
Spectroscopy	Mass spectroscopy	MS (EI, CI, FAB etc), tandem MS, HRMS, ICP-MS, MALDI-TOF	Suitable for organic and inorganic substances. Many different techniques which vary with respect to the ionization method used and the type of sample preparation required. Need to select best method to provide informative fragmentation pattern and molecular ion (if possible).
	Vibrational spectroscopy	FTIR, ATR-FTIR, Vis-NIR fluorescence, Raman, Confocal Raman	Organic and inorganic substances. Several techniques available. Need to ensure peaks in spectrum are sufficiently resolved using appropriate sample preparation and concentration. In IR, key signals above 1500cm^{-1} but comparison of fingerprint region ($\sim 600\text{-}1500\text{cm}^{-1}$) with reference spectra also important. Calibration with known standard is essential.

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	Electronic spectroscopy	UV-Vis, fluorescence spectroscopy	Largely organic substances. Not useful for substances with little or no activity in the UV-Vis region of the spectrum. Usual to quote λ_{max} and extinction coefficient. For comparison with known reference standards, spectra should be run in same solvent to avoid solvatochromic effects (where relevant). Stable baselines must be displayed in all cases.
	NMR	Solution or solid state, various nuclei including ^1H , ^{13}C , ^{19}F , ^{29}Si etc, 1-D, 2-D (COSY, NOESY etc if appropriate)	Largely organic substances. Provides information on chemical composition, formulation, impurity levels. Allows structural elucidation and quantification of components. Internal reference and full integration essential. Peak resolution must be sufficient for unambiguous interpretation and any complex signals expanded for clarity. Proton NMR usually sufficient but ^{13}C (or other nuclei) or 2-D experiments may be required for more complex molecules. Spurious signals must be investigated.
Purity	Separation techniques	GC, GC-MS, GC-MS-MS, GC-ICP-MS, HPLC, LC-MS, LC-MS-MS, LC-NMR, Ion chromatography, GPC, GPC-MS, GPC-NMR	Several different techniques available for both organic and inorganic substances. Essential to confirm the composition and purity. Elution methods should be designed to give optimum resolution of components. Spiking with known standards is useful to help identification. Ideally, impurities should be quantified and characterized individually using at least one other linked technique such as MS or NMR.

SUITABLE ANALYTICAL METHODS FOR SUBSTANCE CHARACTERIZATION

Other analytical methods (as appropriate)	Thermal analysis	TGA, DSC	Melting points and boiling points may be appropriate but usefulness for comparison purposes depends on understanding purity profile
	Morphology	XRD, TEM	Important for solid substances where crystal structure distinguishes the substance from others with the same chemical constitution.
	Biological property measurements	Catalytic activity for enzymes, amino acid sequence	Catalytic activity for enzymes. Must include source organism, substrate and conditions. Amino acid sequence
	Chirality	Polarimetry, LC, capillary electrophoresis, NMR	Important for chiral substances. Several methods to assess optical activity and enantiomeric profile.
	Wet chemistry	Titration (volumetric, potentiometric, complexometric), gravimetric analysis	Solids and liquids but usefulness for comparison purposes may depend on understanding purity profile. Titration of acidic and basic substances may be appropriate
	Microscopy	SEM, TEM, interferometry (surface profiling), SPM, AFM, LM and fluorescence microscopy	Particle size, surface characteristics and substance distribution.
	Materials analysis and analysis of selected physical attributes	Shape, hardness, swelling capacity, density, surface area, refractive index, melting point and boiling point. CE, SEC, MALLS, SEM/TEM for particle sizing, and nanomaterials analysis	Shape, hardness, density, surface area and profile, mineral composition, particle sizing, microscopy etc.

RECOMMENDATIONS:

- All analyses should be carried out on the same batch of material (or a comment added if otherwise prepared by the manufacturer)
- All solvents used for analysis should be spectroscopically pure
- Instruments used for analysis should be calibrated periodically using suitable standards
- If a particular analytical technique cannot be used, justification should be given

Substance characterization MUST include:

- Tests for elemental composition and purity
- For most organic materials, the minimum spectral data package should contain UV-Vis, IR and NMR plus an analytical method such as HPLC or GC. Different techniques may be more appropriate for other types of substances.
- Any of the other analytical methods deemed appropriate to support the case
- Methods capable of identifying specific isomers, eg geometrical or optical isomers, particular salt forms, morphologies or any other defining attribute of the substance where relevant
- Additional information for certain substances, especially UVCBs. Details of raw materials, manufacturing processes, refinement methods, biological species of origin and part of the organism used etc as appropriate. Generic descriptors for minerals. Spectral or chromatographic fingerprints showing characteristic peak distribution pattern.
- Interpretation and verification by a technically qualified individual

ANALYTICAL DOCUMENTATION:

All analyses should display or be accompanied by the following information:

- The substance name, structural formula, the source, the batch number and any other unique identifiers
- The physical form of the substance and its purity in percentage by weight
- The type of test and specific equipment used (manufacturer's make and model)
- The test conditions used, eg temperature, field strength, reference materials, elution methods etc
- The form in which the sample was tested, eg solid, liquid, solution, Nujol mull etc
- Any necessary calibration of the equipment carried out (where appropriate)
- Details of any internal reference standards used, eg TMS in proton NMR spectroscopy, and where they appear in the spectra or test output (where appropriate)
- The number of duplicate tests carried out and whether the result quoted is a mean value (where appropriate)
- A title and a unique reference number on all spectra. All axes must be clearly labelled and the scales indicated. Any scale expansions must be clearly indicated
- The date
- A list of assigned signals in each spectrum plus any relevant integration etc. Any missing or spurious signals must be explained
- The analytical laboratory responsible for performing the test and the name of the analyst (plus head of laboratory)
- Any accreditation or recognized codes of practice used by the testing service provider, eg GLP, 'second person checking' etc
- Any appropriate bibliographic references that support the techniques and methods used or which provide standard spectra for comparison

APPENDIX – Acronyms

AAS	Atomic absorption spectroscopy
AFM	Atomic force microscopy
ATR-FTIR	Attenuated total reflectance – Fourier transform infrared spectroscopy
CE	Capillary electrophoresis
CI	Chemical ionization (mass spectrometry)
COSY	Correlation spectroscopy (NMR)
DSC	Differential scanning calorimetry
EI	Electron impact (mass spectrometry)
FAB	Fast atom bombardment (mass spectrometry)
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
GPC	Gel permeation chromatography
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
ICP-OES	Inductively coupled plasma-optical emission spectroscopy
LC	Liquid chromatography
LM	Light microscopy
MALDI-TOF	Matrix assisted laser desorption/ionization-time of flight mass spectrometry
MALLS	Multi-angle laser light scattering
MS	Mass spectrometry
NIR	Near infrared (spectroscopy)
NMR	Nuclear magnetic resonance spectroscopy
NOESY	Nuclear Overhauser and exchange spectroscopy (NMR)
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy
SEM-EDX	Scanning electron microscopy-energy dispersive X-ray analysis
SPM	Scanning probe microscopy
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
UV-VIS	Ultraviolet-visible spectroscopy
XRD	X-Ray diffraction
XRF	X-Ray fluorescence

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