

Characterization of Multi-constituent Substances for REACH Registration

INTRODUCTION

Under REACH, multi-constituent substances are described as 'well-defined'. This terminology belies the fact that characterization for the purpose of registration can, in some cases, present a significant analytical challenge. The successful analysis of multi-constituent substances requires a combination of in-depth knowledge of the chemical processes used to prepare them and a well-chosen suite of tests.

ECHA's *Guidance for Identification and Naming of Substances* under REACH defines two possible registration routes for multi-constituent substances. They can be registered as reaction masses or, under certain circumstances, the individual constituents can be registered independently¹. Both options necessitate characterization of the multi-constituent substance in order to provide evidence of its composition or to confirm the presence of individual substances within it. This document sets out to define some of the approaches that might be considered, when undertaking this work.

DEFINITION OF A MULTI-CONSTITUENT SUBSTANCE AND IMPLICATIONS FOR CHEMICAL ANALYSIS

ECHA's definition of a multi-constituent substance (summarized below), together with the chemical category of the substance in question, should indicate the required capabilities and scope of any analytical techniques used for characterization.

- It is a reaction mass in which more than one main constituent is present in a concentration $\geq 10\%$ and $< 80\%$ (w/w)
- Constituents present at less than 10% (w/w) are defined as impurities
- All impurities present $\geq 1\%$ (w/w) should be specified by at least one classifier such as name, CAS number etc
- Any substance that may change the hazard classification or PBT assessment of the multi-constituent substance must always be specified as described in the previous point, irrespective of concentration

¹ Another registration option for multi-constituent substances has come to light, and is exemplified by the FARM Consortium's approach to the registration of inorganic NPK fertilizers (www.cms.efma.org). The consortium has elected to define its substances as *preparations* arguing that, irrespective of the manufacturing method used, the final products always contain a combination of a few simple ionic components from a basic, defined set. Consortium members are being recommended to register either the individual constituents of the reaction mass independently, as described above, or to register the starting *materials* used to prepare the substance.

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- The sum of the main constituents plus impurities should be 100% (additives deliberately added to preserve the substance's stability must be taken into account in the mass balance calculation)
- It is the result of a manufacturing process, i.e. it is the product of a chemical reaction
- Impurities are unintended substances present as a result of side-reactions, incomplete reactions or may have originated in the starting materials

The analytical techniques used for multi-constituent substance characterization must have the capacity to distinguish between the various constituents present and to generate direct evidence of their chemical structure and concentration. This includes impurities down to levels of 1% (w/w), or lower in the case of constituents that may impact on the overall hazard classification (CMR, PBT, vPvB). The fact that a multi-constituent substance is defined as the product of a chemical reaction means that detailed knowledge of the process used to prepare it is indispensable both for selecting appropriate analytical tests and interpreting the results.

ANALYTICAL METHODS (a glossary of acronyms is provided at the end of the document)

General points

ECHA's ***Guidance for Identification and Naming of Substances under REACH*** provides very brief details concerning the general spectroscopic and chromatographic techniques that can be used for characterization purposes. In practice, what is sometimes referred to as an 'orthogonal' analytical approach, i.e. one that involves techniques that are independent and mutually reinforcing, is advisable. The analytical categories that are important in such an approach are shown below. It is essential to select those that are ***meaningful*** for each substance.

- **Spectroscopy:** The following four spectroscopic techniques provide the means to acquire structural and compositional information:
 - Mass spectrometry, e.g. EI, CI FAB, electrospray, ICP-MS, MALDI-TOF, tandem MS
 - Structural analysis, e.g. NMR such as ^1H , ^{13}C or other nuclei, COSY, NOESY
 - Vibrational spectroscopy, e.g. FTIR and Raman
 - Electronic spectroscopy, e.g. UV-Vis, fluorescence, atomic absorption/emission

If possible and meaningful, one of each of the above categories of analysis should be included. Clearly, some methods will not be appropriate due to the nature of the substance or its particular chemical functionality.

- **Purity:** A selection of the more important chromatographic techniques that are commonly used for composition and purity determination include the following:
 - Liquid chromatography, especially HPLC
 - Gas chromatography
 - Ion chromatography
 - Capillary electrophoresis
 - Gel permeation chromatography

It is usually sufficient to employ just one of the above chromatographic techniques providing the purity profile can be adequately demonstrated. Note that for multi-constituent substances, integrated separation-analysis methods are the most informative (see below).

- Where **additional substance identifiers** are useful for characterization, some of the following techniques may also be applicable:
 - Elemental analytical techniques, e.g. ICP-OES, EDX, wet chemistry, microanalysis
 - Thermal analysis, e.g. DSC and TGA
 - Assessment of morphology or other physical attributes, e.g. XRD, particle size, surface properties, SEM

ANALYTICAL STRATEGIES

Depending on the chemical nature and complexity of the multi-constituent substance, there are two key strategies that can be considered:

- Simultaneous separation-analysis techniques
- Analysis of the reaction mass without physical separation of the constituents

Knowledge of the purity profile of the starting materials is important since it may explain the origin of any unknown components of the multi-constituent substance. For the same reason, any standard reference samples used for the purpose of 'spiking' during analysis, should also be examined.

In situations where the physical nature of a substance inhibits the characterization process, chemical derivatization prior to analysis may help to overcome the problem.

A preliminary analytical screen can provide a cost effective view of the complexity of each individual case and define the specific techniques needed for full characterization.

Simultaneous separation-analysis

Techniques that offer simultaneous separation and analysis, the so-called 'hyphenated' techniques, have the potential to make a significant contribution to the characterization process and require the least amount of sample handling. The table below shows a selection of typical chromatographic and spectroscopic techniques that are often integrated.

Chromatographic Techniques	Spectroscopic Techniques	Hyphenated Techniques
LC	MS	GC-MS
HPLC	UV-Vis	CE-MS
GC	FTIR	LC-LC-MS
CE	NMR	HPLC-NMR
IC	ICP-OES	LC-SPE-NMR
SPE		GC-FTIR
GPC		CE-UV
		LC-NMR-MS
		IC-MS

Analysis of the reaction mass **without** physical separation of its constituents

There are several reasons why this approach may be adopted in the characterization process: Physical separation may be difficult or it may not be possible to provide unequivocal evidence to support the composition of the substance using certain analytical strategies. The most likely reason is that physical separation is simply unnecessary since sufficient data can be obtained from the multi-constituent substance as a whole. The use of XRD, ICP and IR to reveal the full identity of many crystalline, inorganic substances is a good example to illustrate this point. The non-separation approach may involve the use of spectral fingerprints, if the relevant signals can be sufficiently resolved and identified. Care is needed when comparing fingerprints with reference spectra in data libraries since these may have been acquired using different instruments, conditions or methods.

ORGANIC AND INORGANIC MULTI-CONSTITUENT SUBSTANCE ANALYSIS

For many organic multi-constituent substances, a substantial level of characterization can usually be achieved using one or more of the hyphenated techniques described above. Even without physical separation, techniques such as NMR can yield a considerable amount of structural and compositional information. Further analysis may still be necessary to complete the full assessment. Whilst there are no typical examples, for illustrative purposes, an analysis might reasonably be expected to include HPLC- or GC-MS, ^1H and possibly ^{13}C NMR, FTIR, UV-Vis. Any free acids can be assessed by titration whilst salts may call for ion chromatography or specific chemical analysis. Organic solids such as pigments may have characteristic powder XRD patterns or thermal profiles as assessed by DSC/TGA.

The characterization of inorganic multi-constituent substances is likely to rely heavily on XRD if the materials concerned are crystalline. Ion chromatography can be useful for the identification and quantification of specific cations and anions. Elemental analysis can be achieved using atomic absorption or emission spectroscopy and ICP-MS/ICP-OES. The latter techniques are particularly useful for identifying metallic elements and ICP-MS can also be useful for defining metal speciation. Important spectroscopic techniques include IR, Raman and NMR (solution or solid state) of particular nuclei such as ^{31}P . Thermal analytical techniques such as DSC and TGA can be useful for identifying characteristic melting/sublimation points, phase transitions and decompositions. Many chemical analytical methods have been developed for measuring specific constituents and can distinguish between, for example, nitrate nitrogen and ammoniacal nitrogen.

ADDITIONAL INFORMATION

In addition to the actual analytical testing, it is important to make use of all the available information that might help to predict or define the **theoretical** composition of the substance. This should include details of the chemical process(es) involved in manufacture, the starting materials used, their stoichiometry and respective purity profiles, and any known side-reactions. This not only helps to define the most appropriate analytical techniques to apply but also facilitates the interpretation of results. Any other relevant information that could strengthen the case should be added.

Annex VI of the REACH Regulation says that a '***description of the analytical methods or the appropriate bibliographical references.....sufficient to allow the methods to be reproduced***' should be provided. It is advisable to include unique substance identifiers, equipment, conditions and methods used, clearly labelled and interpreted spectra, and details of the analytical laboratory responsible for the testing together with any accreditation they may have.

Some SIEFs and consortia have developed registration strategies that include details of specific analytical methods for substance characterization in order to facilitate sameness checking. It is sensible to take advantage of any such guidance that may be available.

APPENDIX – Glossary

AAS	Atomic absorption spectroscopy
AES	Atomic emission spectroscopy
CE	Capillary electrophoresis
CI	Chemical ionization (MS)
COSY	Correlation spectroscopy (NMR)
DSC	Differential scanning calorimetry
EDX	Energy dispersive X-Rays
EI	Electron ionization (MS)
FAB	Fast atom bombardment (MS)
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
GPC	Gel permeation chromatography
HPLC	High performance liquid chromatography
IC	Ion chromatography
ICP-MS	Inductively coupled plasma-mass spectrometry
ICP-OES	Inductively coupled plasma-optical emission spectroscopy
LC	Liquid chromatography
MALDI-TOF	Matrix assisted laser desorption/ionization-time of flight mass spectrometry
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser and exchange spectroscopy
SEM	Scanning electron microscopy
SPE	Solid phase extraction
TGA	Thermogravimetric analysis
UV-VIS	Ultraviolet-visible spectroscopy
XRD	X-Ray diffraction
XRF	X-Ray fluorescence

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