Substance characterization for UVCBs
Part 1: Organic Substances

INTRODUCTION

UVCB substances, by their very nature, can present a difficult analytical challenge and their characterization demands a somewhat different approach. Formal substance identification requires not only the main identifiers (name, source and production process) but also what ECHA's guidance documents refer to as 'other identifiers'. This term primarily covers whatever appropriate, measurable information can be used to characterize the particular category of substance. It may include, among other things, chromatographic/spectral fingerprints, physical parameters such as boiling range, enzyme activity and the concentration of specific constituents such as metals.

Substance identity is frequently defined by the commercial sector within which each UVCB is produced and marketed. Formal quality standards are usually set that must be fulfilled in order for a substance to be sold for a given application. Typically, these standards form the basis of product grading and, ultimately, pricing.

Many large consortia and trade associations have already defined excellent analytical strategies for the UVCBs that fall within their commercial area of interest. However, not all registrants benefit from being under the auspices of such groups and must devise their own approach to substance characterization.

In this, the first of two articles, the design of a potential analytical strategy for the characterization of ***organic*** UVCBs is discussed. A second paper, focussing on inorganic UVCBs, will follow shortly.

Designing an appropriate analytical strategy

Chapter 4 of ECHA's ***Guidance for identification and naming of substances under REACH*** provides a number of useful technical recommendations for UVCB characterization. Chapter 4.3.1.1, for example, states that the chemical composition and identity of the constituents should be given ***as far as known***. This information can be provided in a generic way if appropriate, e.g. the carbon chain-length range, or information on composition can be derived by comparison with reference samples. Typical concentrations and concentration ranges need to be given and unknown constituents should be identified using a generic description of their chemical nature if this is possible.
The following key points are made:

- For cases where spectral data provide information on the composition of the UVCB substance, such information should be given.
- Chromatographic or spectral images that show a characteristic peak distribution pattern, i.e. 'fingerprints' can be used.
- Valid constituent separation techniques might be used where appropriate.

- The chemical composition and identity of the constituents should still be given as far as known. Information on chemical composition can be given on the basis of well-known reference samples and standards.
- '…. .it is the responsibility of the registrant to present appropriate spectral data.'

Using this Guidance, it is possible to develop an understanding of the nature and detection capabilities of the analytical techniques required in order to generate the necessary data for each individual substance.

In addition to the practical aspects of characterization, it is highly likely that published scientific literature and resources such as spectral libraries will play an important role. Examples of the latter include the National Institute of Standards and Technology (NIST) 08 Mass Spectral Library[1], the SpecInfo data collections available from Wiley-VCH (NMR, IR and MS)[2] and the Aldrich Library of ^{13}C and ^{1}H FT-NMR Spectra and FT-IR Spectra[3]. Commercial crystallographic data libraries may also be important, e.g. the Joint Committee on Powder Diffraction Standards (JCPDS) database from the International Centre for Diffraction Data (ICDD)[4]. There are often more specialised reference libraries associated with some of the larger UVCB groups.

The following example aims to demonstrate how a combination of analytical techniques and existing reference materials might be used for the characterization of complex plant-derived essential oils such as peppermint, rose, and citrus oils. As a general strategy, it could be applied to other organic UVCBs but clearly, measurements of a more substance-specific nature will need to be considered and selected accordingly.

Example: ***Plant-derived essential oils***

Essential oils primarily comprise a complex combination of volatile substances of terpenoid and non-terpenoid origin. These include representatives from a range of chemical classes such as alcohols, acids, esters, epoxides, aldehydes, ketones, amines and sulphides. Some essential oils can contain literally hundreds of different components that span a wide concentration range. However, in many cases, a relatively small number (perhaps 20-30) make up in excess of 90% of the total oil and in some instances, individual components can account for in excess of 50%.

There is a considerable body of literature concerning the analysis of essential oils, especially those for which there is a large commercial market, such as citrus oils. The composition of an essential oil defines its quality, function and, ultimately, its market value. Not surprisingly, there exist a number of specific protocols for analysing and assaying the substances that fall within this group. Some were developed for use as quality control measures for the flavours and fragrances industry and are used, for example, to detect the adulteration of higher quality oils with less expensive ones. These should certainly be considered when designing a strategy for substance characterization.

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[1] http://www.nist.gov/srd/nist1.htm

[2] STM Data, Wiley-VCH, SpecInfo data collections www.wiley-vch.de/stmdata/specinfo.php

[3] Aldrich Library of 13C and 1H FT-NMR Spectra, C. Pouchert, Wiley, 2009 and The Aldrich Library fof FT-IR Spectra, 2nd Edition, C. Pouchert, Wiley, 2009

[4] See http://www.icdd.com/

The ‘grey literature’ is another potentially useful resource. The technical publicity material produced by many analytical equipment manufacturers to illustrate the applicability of a particular instrument for specific markets can be very useful. Such material often includes a reasonable level of experimental detail and may also be supported by relevant references providing further information.

Listed below are several mainstream analytical techniques that are useful for generating both spectroscopic and chromatographic data. It goes without saying that it is the integrated techniques that provide the greatest level of information and hence the most comprehensive degree of substance characterization. In addition to the mainstream techniques, a selection of more specific methods, recognized as being important for the assessment of essential oils, is also given. Such tests and measurements generally provide data that fall under the heading ‘other identifiers’.

1. Chromatography
    a. Gas chromatographic techniques
    b. Liquid chromatographic techniques

2. Spectroscopy
    a. ^{1}H and ^{13}C NMR spectroscopy
    b. FT-IR and Raman spectroscopy
    c. UV-visible spectroscopy

3. Other techniques
    a. Refractive index measurement
    b. Polarimetry
    c. Specific gravity measurement
    d. Titration
    e. Solubility in alcohol
    f. Evaporation residue

Gas chromatography (GC) is used extensively for the quantitative and qualitative analysis of essential oils, especially capillary GC with flame ionization detection (FID) and GC with mass spectrometry (GC-MS). Peak assignment, certainly for the main components, can often be achieved through analysis of authentic reference samples under identical experimental conditions. Computer matching using MS libraries such as NIST 08 (Ref. 1) and comparison of fragment patterns with those in the published literature are also important. More detailed analyses have been achieved using high resolution GC-MS with fragment isotope ratio analysis[5], 2-D GC-FID[6], and chiral GC[7].

The non-volatile constituents in essential oils can be identified by liquid chromatography, especially high performance liquid chromatography-MS (HPLC-MS) with UV spectroscopy of the individual peaks where this is possible.[8] Again, comparison with reference standards is important for the verification process.

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[5] A. Satake,K. Furukawa, T. Ueno and H. Ukeda, ***Biosci. Biotechnol. Biochem***., 2004, **68** (**2**), 312-316.

[6] See P. T. Stevens, LECO Corporation, 2007 http://www.leco.com/resources/application_note_subs/pdf/separation_science/-314.pdf

[7] M. Eleni, M. Antonios, K. George, S. Alexios-Leandros ans M. Prokopios, ***Molecules***, 2009, **14** (***2***), 839-849

[8] See for example J. A. Manthey and B. S. Buslig, ***Proc. Fla. State Hort. Soc***., 2003, **116**: 410-413; and http://www.leco.com/resources/application_note_subs/pdf/separation_science/-289.pdf

^{13}C NMR spectroscopy is seen as a complementary (or even an alternative) technique for the analysis of essential oils[9]. The spatial arrangement of the signals allows substantial structural elucidation ***without*** prior separation of the individual components. Computer aided techniques[10] have been developed to facilitate the interpretation of ^{13}C NMR spectra and have also been used in conjunction with GC-MS to identify essential oil constituents. [11]

Near infrared spectroscopy has been used to determine the components of various citrus oils[12] and has again been developed for product authentication and detection of adulteration.[13] Characteristic signals, attributable to key constituents such as limonene, can be distinguished using both FT-IR and NIR-FT Raman spectroscopy and can be used for quantitative differentiation between individual oil types.[14] Raman spectroscopy itself can be employed as part of the quality control process. [15]

UV-visible spectroscopy can be informative, particularly in conjunction with other characterization techniques. Published absorption values exist for essential oils and the relative intensities of the peaks in a given spectrum provides additional supporting information. [16]

The analysis of essential oils also involves the determination of several other properties.[17] The specific gravity (by pycnometry) and refractive index are usually measured and compared with known standards. Polarimetry, to determine the optical rotation, gives information that is highly characteristic of the individual oil type. In some cases, the various constituents are present as a racemic mixture but in others, there may be a preponderance of one particular optical isomer. In practice, this means that the degree of net rotation will vary from oil to oil making verification by comparison with known standards essential. There are several titration-based techniques that are often used, an important example being the quantification of the total aldehyde content by hydroxylamine or acid/base titration. Colorimetric measurements are used to determine the quantity of volatile esters present after conversion to the corresponding hydroxamic acids and complexation with ferric chloride. Other analyses include the measurement of the evaporation residue, the solubility in 90 and 95% ethanol,[18] and the iodine, acid and saponification values.

To facilitate the interpretation of spectral and analytical data, specialised spectral libraries are available. The text on essential oils analysis mentioned earlier (Ref. 9) contains analytical data for 60 of the most commercially important essential oils. Other specialized reference works, offer comprehensive information on analytical techniques and also provide good reference data.[19]

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[5] Essential Oils Analysis by Capillary Gas Chromatography and Carbon-13 NMR Spectroscopy, 2nd, Completely Revised Edition, K.-H. Kubeczka and V. Formácek, Wiley, 2002

[10] F. Tomi and J. Casanova, ***Acta Hort***. (ISHS), 2006, **723**, 185-92

[11] J. W. Alencar, M. G. de Vasconcelos Silva, M. I. L. Machado, A.A. Craveiro, F. J. de AbreuMatos and R de Abreu Magalhães, ***Spectroscopy***, 1997, **13** (***4***), 265-273

[12] B. Steuer, H. Schulz and E. Läger, ***Food Chemistry***, 2001, **72** (***1***), 113-7

[13] H. R. Juliani, J. Kapteyn, D. Jones, A. R. Koroch, M. Wang, D. Charles and J. E. Simon, ***Phytochemical Analysis***, 2006, **17** (***2***), 121-8

[14] H. Schulz, B. Schrader, R. Quilitzsch and B. Steuer, ***Applied Spectroscopy***, 2002,**56** (***1***),117-124

[15] K. R. Strtehle, P. Rösch, D. Berg, H. Schulz and J. Popp, ***J. Agric. Food Chem***., 2006, **54** (***19***), 7020-26

[16] Food Chemicals Codex, 5th Edition, Institute of Medicine (US) Committee on Food Chemicals Codex, 2003, National Academy Press

[17] T. Gentry, ***Current Protocols in Food Analytical Chemistry***, 2002, G1.5.1-G1.5.24, John Wiley and Sons Inc.

[18] M. M. Ahmad, S.-Ur-Rehman, F. M. Anjum and E. E. Bajwa, ***Int. J. Agri. Biol***, 2006, **8** (***2***), 186-90

[19] See for example, Citrus Oils: Advanced Analytical Techniques, Composition and Biological Activity, G. Dugo and L. Mondello, 2010, CRC Press; Citrus Processing: A Complete Guide, D. A. Kimball, 1999, 2nd Edition, Springer. See also reference 16.

Summary

The analysis of essential oils has been used to demonstrate how an organic UVCB substance can be characterized using a combination of standard techniques and an appropriate set of secondary tests. For other organic substances, the choice of secondary tests will obviously vary but it is always good practice to select those tests that are supported by good reference material or published standards for the comparison and validation of experimental data.

All experimental work (and reference materials used) should be fully documented in a way that is 'sufficient to allow the methods to be reproduced' (Annex VI, 2.3.7).