# Testing of the quality of essential and vegetable oils

SAEOPA
Southern African Essential Oil Producers' Association

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Department:
Trade, Industry and Competition
REPUBLIC OF SOUTH AFRICA

Schweizerische Eidgenossenschaft
Confédération suisse
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Confederaziun svizra

Swiss Confederation

Federal Department of Economic Affairs,
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UNIDO
UNITED NATIONS
INDUSTRIAL DEVELOPMENT ORGANIZATION
Progress by innovation

GQSP
GLOBAL QUALITY AND STANDARDS PROGRAMME

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# List of acronyms

| | |
|---|---|
| **ABioSA** | ABS Compliant Biotrade in South(ern) Africa |
| **ABS** | Access and benefit-sharing (for biotrade value chains) |
| **AOAC** | American Organization for Analytical Chemists |
| **AOCS** | American Oil Chemists Society |
| **ASTM** | American Standards and Test Methods |
| **BP** | British Pharmacopoeia |
| **CLP** | Classification, labelling and packaging (of chemicals) |
| **CRM** | Certified reference material |
| **EC** | European Commission |
| **EP** | European Pharmacopoeia |
| **UNECE** | United Nations Economic Commission for Europe |
| **FFA** | Free fatty acid |
| **FID** | Flame ionization detector |
| **GC** | Gas chromatography |
| **GHS** | Globally Harmonized System (for the labelling of chemicals) |
| **GIZ** | Deutsche Gesellschaft für Internationale Zusammenarbeit |
| **GLP** | Good laboratory practice |
| **HPLC** | High-performance liquid chromatography |
| **IEC** | International Electrotechnical Commission |
| **IFEAT** | International Federation of Essential Oils and Aroma Trades |
| **ILAC MRA** | International Laboratory Accreditation Cooperation Mutual Recognition Arrangement |
| **ISO** | International Organization for Standardization |
| **ISO TC 54** | ISO Technical Committee for Essential Oils |
| **LC** | Liquid chromatography |
| **MS** | Mass spectroscopy |
| **MSDS** | Material data safety sheet |
| **OECD** | Organization of Economic Cooperation and Development |
| **PIF** | Product information file |
| **REACH** | Registration, Evaluation, Authorization and Registration of Chemicals regulation |
| **SAEOPA** | South African Essential Oils Producers' Association |
| **SECO** | Swiss State Secretariat for Economic Affairs |
| **UN** | United Nations |
| **UNIDO** | United National Industrial Development Organization |
| **USP** | United States Pharmacopeia |

# 1 Introduction

It is widely accepted that Southern Africa has many indigenous plants producing essential and vegetable oils that have potential commercial value. However, to exploit these commercially on an international scale, the industry needs to ensure sustainability of supplied products at consistent quality and in viable quantities.

Essential oils are the fragrant essences of plants in their purest and most concentrated state. They are primarily composed of terpenes and their oxygenated derivatives. The oils can be obtained from different parts of the plant, such as flowers, roots, bark, wood, seeds or leaves; and may be extracted in several ways, such as steam distillation, hydro distillation, or solvent extraction. They are distilled and used in personal and home fragrances, cosmetics, food and beverage flavouring, and the health sector. Oils from the same plant can vary from supplier to supplier because of many factors, such as the parts of the plant used, ground conditions, weather, geographical location, and the level of expertise and care taken by the farmers and distillers of the oil.

For the industrial application of these products, the requirement is for consistent, standardised essential oils that do not vary from what is deemed to be acceptable for such products. There is also a need to check the quality of the oils for possible contaminants or evidence of spoilage or adulteration.

The industry needs the support of a reliable quality infrastructure to provide testing, traceability and assurance of competence to deliver assurances that are necessary to support access to global markets for these oils and to support the formulation and production of products using locally produced and imported oils.

Due, in part, to the relatively niche nature of the essential oils market, there is a lack of testing facilities in South Africa presenting themselves as specialists in this field, and that serve the needs of this market exclusively. There are several tests that can be conducted on samples of essential oils to ascertain different quality facets of the product, each of which is used to support different regulatory or customer-directed requirements. Many of them fall within the areas of competence of several laboratories, but there remain some analyses, required for special applications, for which the required competencies are not very common.

Suppliers, buyers and regulators are increasingly faced with the challenge of having to either commission testing or demand that testing is carried out, but are not clear as to what tests, studies or analyses are required, and what to demand of the bodies conducting those tests for the results to be acceptable.

This report seeks to cover the common tests performed on essential and vegetable oils, and why these may be required. It also seeks to understand some of the challenges unique to these niche products, and the typical institutions that perform these tests and analyses.

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# 2 Testing requirements for essential oils and vegetable oils

Essential oils are widely used in the food and fragrance industries and, to a lesser extent, in the cosmetics industry, and also in fields related to health, such as pharmaceuticals, aromatherapy and phytotherapy[1]. There are about 300 different essential oils that are well characterized and commonly used. This number continues to increase as new botanicals are discovered and tested.

> *A worldwide estimation of production of essential oils is a very hard task because of the huge number of small farmers for many of the products and the complexity of (local/domestic) existing supply chains, always considering second or third hand information, extrapolations, guesses and estimates, etc., thus, there are "guestimates" with a margin of deviation ± 25%. Taking all this into consideration, and according to information provided by IFEAT, data obtained in 2018 from different sources indicate a world production of 150.000 – 175.000 tonnes resulting in 2.750.000 – 3.250.000 Million US dollars[2].*

To support trade in these important products at the international level, ISO's Technical Committee for Essential Oils (ISO TC 54) lists as its mandate the development of international standards covering:

- Elaboration of specific monographs for quality standardisation of every essential oil.
- Standardisation of analytical methods to control essential oils' quality.
- Standardisation of requirements for transport, labelling and marking of essential oils.
- Standardization of the botanical names of plants used for the production of the essential oils.

As is evident from the above list, trade and other commercial applications of these oils rely on claims of conformity to certain parameters, which claims are backed up by evidence provided by some form of analysis performed in a laboratory. The products are subject to a range of different tests depending on the type of claims made and the reason for making those claims. The tests include:

- Physical testing.
- Chemical testing.
- Organoleptic testing.
- Performance testing.

*1 Source: ISO TC 54 Technical Committee business plan – Essential Oils www.iso.org*

*2 Source: Extracted from the ISO TC 54 Technical Committee business plan – Essential Oils www.iso.org*

The reasons for the testing also vary. They can range from the intrinsic safety of the product as containing chemical compounds that need to be classified in terms of the associated risks which, in turn, determine how they are labelled, packaged, handled and stored. The products are used in various applications in food, beverages and cosmetics, and in terms of the governing legislation for products and ingredients used, they are required to undergo tests to demonstrate the safety and suitability for their use in the production of food, cosmetics, and healthcare-related commodities.

Classes of foodstuffs and cosmetic products are those where claims of one or more performance attributes are made and, in such cases, under both cosmetics and foodstuff regulations as well as advertising standards, such claims are required to be supported by independent studies or analyses. Additionally, if the claims are related to health benefits, the studies would need to adhere to the guidelines related to the registration of medicines and approval would have to be sought from the relevant medicines control authority.

## 2.1 Substances of concern

Essential oils and vegetable oils are extracted from plant material, and several compounds associated with plant and plant products can therefore appear as artifacts in these preparations. These include:

- Microbiological load, or the presence of microbiological contaminants, which can either spoil the product or pose a health risk to the user of the product.
- Toxins, such as aflatoxins, associated with microbiological contamination of the plant material at any stage during its collection, production, and extraction.
- Pesticides, residues of pesticides and heavy metals, which could appear because of inorganic fertilizers used.

Chemical analysis for the identification and quantification of these substances may be a prerequisite for the importation of essential oils into certain countries, requiring a certificate of analysis from a formally recognized competent body (notified body under European Union (EU) regulations).

## 2.2 Product quality

In commercial applications, essential oils may be tested to determine their quality and purity by the presence and relative concentrations of constituent aromatic compounds in the product.

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### 2.2.1 Standards

Several essential oils that are widely used industrially are well characterized, and these have been published as international standards and monographs. ISO TC 54 has published some 136 international standards, many of which are product standards that characterizing widely used essential oils. Several product standards have also been published in pharmacopoeia, such as the British Pharmacopoeia (BP), the European Pharmacopoeia (EP), and the United States Pharmacopeia (USP). All these standards have been developed through extensive international stakeholder participation, international guidelines were followed to validate the requirements, and they consequently are regarded as international benchmarks for those products. It is customary practice to claim quality of an essential oil relative to one of these international standards.

The monographs typically list a series of physical and chemical tests to be performed, and the acceptance criteria for each serve to determine if the product conforms to the requirements when examined.

# 2 Testing requirements for essential oils and vegetable oils

## Special testing

### Oxidation and chemical degradation because of spoilage

Over and above the constituents of the oil, the quality of the product is also affected by the chemical degradation of oils and other elements in the product. In conjunction with the somewhat subjective organoleptic testing to detect rancidity in the oils, chemical testing of the peroxide values and the presence of methyl ester is routinely done to determine freshness of the product.

### Adulteration

Some essential oils are rare and are consequently sold at premium prices. Unfortunately, this can be exploited by unscrupulous suppliers who present oils that are not pure, as the genuine product, and charge a premium. Cases of dilution of essential oils with inert carriers or the substitution of oils with alternative oils or ingredients with similar profiles, have been detected.

In cases where adulteration is suspected, the standard range of tests may not always detect all adulterations and further, more advanced analysis may be required. These tests include the use of gas chromatography with mass spectrometer as a detector, and advanced chiral specific gas chromatography columns to separate, identify and quantify the relative concentrations of different enantiomers of the constituent compounds that appear in the sample.

### Claims of origin

As with the problem of adulteration, some suppliers also make false claims related to the origin of the plant material from which the essential oil being offered, was derived. These can range from claims related to organic production methods, to the region in which the plants were grown and harvested. Again, advanced chromatographic techniques can be employed using both gas and liquid chromatography, coupled to a tandem mass spectrometer (GC MS MS) or (LC MS MS), which is particularly sensitive and can detect compounds present in very low concentrations. Mass spectroscopy is a particularly sensitive detection method that allows products to be identified based on their spectral patterns, which can be matched to those in published spectral databases. While reliable identification can be made using this technique, when compounds are in very low concentrations the challenge arises to separate the molecules to allow identification. New techniques are being developed all the time in this area and the compounds that can be detected and the limits at which they can be detected, are being constantly re-evaluated.

# 3 Chemical safety

The safety of chemical products forms the basis of most regulations related to chemical products.

## 3.1 European Union's (UN) mandatory requirements

Products must comply with the EU's mandatory legal requirements for natural ingredients for cosmetics. These include:

- **Cosmetic Regulation (EC 1223/2009)**. This is the central regulatory framework for cosmetic products for the European market, covering the safety and effectiveness of cosmetic products. It is especially advisable to focus on Chapter 3: Safety Assessment, Product Information File.
- **Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation**. This regulation is adopted to protect human health and the environment from the risks posed by chemical products. It places the burden of proof on companies introducing any compounds or preparations into the market, to demonstrate that they are safe, and to provide information on their safe use, storage and disposal. The regulation promotes the use of alternate methods of determining safety and preparations or mixtures to reduce the number of tests on animals.
- **EU Commission Regulation (EU) No 655/2013**. This regulation requires claims for a cosmetic product (explicit or implicit) to be supported by sufficient and provable evidence.
- The EU has packaging and labelling requirements for chemicals based on the **Globally Harmonised System of Classification and Labelling of Chemicals (GHS)**, outlined in its Classification, Labelling and Packaging Regulation (EC) 1272/2008.

According to the Classification, Labelling and Packaging regulation, labelling should include the following:

- The name, address and telephone number of the supplier.
- The nominal quantity of a substance or mixture in packages made available to the general public (unless this quantity is specified elsewhere on the package).
- Product identifiers.
- Where applicable, hazard pictograms, signal words, hazard statements, precautionary statements and supplemental information required by other legislation.

The safety of chemical products forms the basis of most regulations related to chemical products.

# 3 Chemical safety

## 3.2 Classification, labelling and packaging (CLP)

The EU's regulations on the classification, labelling and packaging of chemical products and mixtures have been in effect since January 2009. The regulations are based on the United Nations' Globally Harmonized System (UN GHS).

The classification and labelling of all chemical products is governed by the inherent safety characteristics of the product which, in turn, determine how the product is stored, handled and labelled. It also provides information on the inherent hazards and related precautionary measures, such as the safe exposure limits, protection information, health hazard information, emergency and first-aid procedures, and the spills, leaks and disposal procedures to be followed.

These tests are the subject of a related information brochure prepared by the Southern African Essential Oils Producers' Association (SAEOPA) with the assistance of the United National Industrial Development Organization (UNIDO). A copy of this material is available on the SAEOPA website[3].

These tests include the following essential tests:

- Melting point.
- Boiling point.
- Relative density.
- Vapour pressure.
- Water solubility.
- Partition coefficient.
- Flash point.
- Flammability.
- Explosive properties.
- Self-ignition temperature.
- Oxidizing properties.

These methods are well established, and performed by many analytical chemistry laboratories. The reference test methods for these parameters are listed in Table 1.

| MELTING POINT | BOILING POINT | RELATIVE DENSITY | VAPOUR PRESSURE | SURFACE TENSION | WATER SOLUBILITY | PARTITION COEFFICIENT |
|---|---|---|---|---|---|---|
| OECD 102 | OECD 103 | OECD 109 | OECD 104 | OECD 115 | OECD 105 | OECD 107<br>OECD 117<br>OECD 123 |
| Method A.1 (EC 400/2008) | Method A.2 (EC 400/2008) | Method A.3 (EC 400/2008) | Method A.4 (EC 400/2008) | Method A.5 (EC 400/2008) | Method A.6 (EC 400/2008) | Method A.8 (EC 400/2008) |
| **FLASH POINT** | **FLAMMABILITY** | **EXPLOSIVE PROPERTIES** | **SELF-IGNITION TEMPERATURE** | **OXIDIZING PROPERTIES** | | |
| Method A.9 (EC 400/2008) | Method A.12 (EC 400/2008) | Method A.14 (EC 400/2008) | Method A.15 (EC 400/2008) | Method A.17 (EC 400/2008) | | |

*Table 1: Table of standard test methods used for the registration of chemical products based on the UNECE GHS*

In 2021, the ABS Compliant Biotrade in South(ern) Africa project (ABioSA), funded by the Swiss State Secretariat for Economic Affairs (SECO) and implemented by the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) developed best practice guides[4,5] to help small businesses understand what tests and analyses are required in order for vegetable, seed, and essential oils in cosmetic products to comply with the EU's CLP, REACH and cosmetics regulations.

**3** *https://www.saeopa.co.za/news/guide-eu-regulation-on-classification-labelling-packaging-for-natural-ingredients/*

**4** *https://www.abs-biotrade.info/fileadmin/Downloads/1.%20PROJECTS/ABioSA/Repository/ABioSA_Guides_August_2021/ABioSA-guide-Tests-EU-compliance-Minimum-analysis-required-for-oils-and-cosmetic-products-2021.pdf*

**5** *www.abs-biotrade.info/fileadmin/Downloads/1.%20PROJECTS/ABioSA/Repository/ABioSA_Guides_August_2021/ABioSA-guide-Classification-Labelling-and-Packaging-CLP-2021.pdf*

# 4 Other regulatory requirements

Essential oils that are used in cosmetics or cosmetic applications need to also demonstrate conformity to a series of mandatory requirements. For the purposes of this document, the requirements of the EU are provided, as a substantial portion of the trade in these products is conducted with companies in the EU, and many other countries and regions have adopted similar provisions in their own legislation – even if these differ slightly, similar provisions are applicable. It is worth noting that the South African Department of Employment and Labour promulgated the Regulations for Hazardous Chemical Agents in 2021, which makes compliance with the GHS mentioned above mandatory in South Africa.

## 4.1 Novel products

All ingredients used in cosmetics and foods need to be approved for use by the designated regulators. This approval is based on the presentation by the supplier or applicant of evidence that successful trials had been conducted by a registered facility that complied with the appropriate Good Laboratory Guidelines (GLP). Facilities conducting these trials or studies are registered by the regulators to follow appropriate protocols and other provisions in the foodstuffs and cosmetics regulations related to such studies.

In general, these studies look at two aspects of the product or ingredient, namely its safety and efficacy. For safety studies, products are tested for a number of possible negative effects that could result from the intended or unintended use of the product, including aspects such as toxicology, skin sensitisation, and possible neurotoxicity. These tests used to be conducted on animals, but alternatives to animal testing have been developed and are being used of late.

In general, these studies look at two aspects of the product or ingredient, namely its safety and efficacy.

Increasingly, essential oils are used for their possible positive benefits in products, ranging from the repelling of insects to the reduction of pain. Any claims to such affect by the suppliers of those products would need to be substantiated by studies that verify the ability to produce the claimed result (or efficacy). For the most part, claims will be regulated by legislation related to advertising standards, but if the claims relate to possible health benefits, the efficacy studies would have to be conducted by an approved facility (see comments on GLP studies in section 9.2), and submitted to the relevant health products regulatory authorities before the products could be approved and registered.

New registrations of products to be used in foods or cosmetics will therefore be based on a full characterization of the product or the material safety data sheet (MSDS), and the results of the study. Once approved, ingredients are then added to the list of approved ingredients by the relevant regulator (foodstuffs, cosmetics and disinfectants regulator), and are deemed to be safe for use in subsequent formulations provided subsequent batches of the ingredient conform to the standards used to characterize them during registration.

## 4.2 Essential oils as ingredients

The suppliers of cosmetics products are required to maintain a full product information file (PIF) for each batch of the product produced, which includes all ingredients used, their batches, and the MSDS for each.

Suppliers of essential oils therefore have to furnish a MSDS for the products, as well as the quality parameters described in recognized national or international standards or monograms.

# 5 The quality of essential and vegetable oils

Essential oils and vegetable oils are produced from natural substances (plants), and can be obtained from all over the world.

The oils are typically the concentrated extracts of plants, which are used in various applications across several industries. The oils often have a stronger smell than the plants from which they are derived, and may be used as a perfume or fragrance based on the aromatic compounds present in the oils. The application and registration of these products are regulated by the provisions related to fragrances in the foodstuffs and cosmetics regulations in most countries.

The aromatic compounds themselves may also exhibit desirable or undesirable qualities that can be exploited in diverse ways. These qualities include anti-microbial effects, anti-oxidative effects, topical irritation, allergenicity, and other effects related to inhalation or topical exposure.

Products are tested at various stages during the production and marketing of the product. The quality of the extraction or distillation is often monitored by one or more physical, chemical or organoleptic tests.

Manufacturers prepare extractions of plant materials to obtain the essential oils or preparations that are provided to the market. Several extraction techniques can be used depending on the base material and the desired products. Essential oils are generally produced by steam extraction of the desired parts of the plant. Cold pressing of the plant material is the most commonly used method for the production of vegetable oils.

## Carrier oils

The aromatic nature of the essential oils means that some of the extractions can be volatile and unstable, or the activities can be more intense than desired, causing the compounds to cause irritation or burning in a cosmetic application. In these cases, carrier oils that are less volatile are used in preparations containing essential oils, and to stabilize and dilute the essential oils. Carrier oils are typically extracted from the seeds, kernels or nuts of plants, and include products such as avocado, baobab, coconut or marula oil.

Products are tested at various stages during the production and marketing of the product. The quality of the extraction or distillation is often monitored by one or more physical, chemical or organoleptic tests. In some cases, producers may be required to produce independent test reports to either regulators or buyers to provide them with the assurance that the product meets their requirements.

Because these products are used in the cosmetics industry, the products are well characterized during registration, and the purity and quality of the preparations are normally determined by a series of chemical and physical tests that define the profiles for these oils.

The history of the fragrance industry goes back many years and, in the past, dealers often obtained the oils from farmers or wholesalers whose practices they came to trust over time. These "collegial" relationships may not provide the best deal for all parties, and can pose significant challenges for new entrants into this field.

As new suppliers enter the market and supply chains are challenged by price and other factors, the industry (suppliers and buyers alike) is starting to turn towards independent and objective ways of demonstrating the authenticity, stability and quality of the product concerned.

# 6 Purity (against claims)

There is an estimated 300 different commercially traded essential oils. Each oil has unique characteristics that make it desirable, and consequently marketable, and differentiate it from other products.

The product standards cover several parameters that can be used to characterize the oil and serve to indicate its purity. Samples are tested relative to the quality parameters published in the standards, and if they fall within the limits provided in the documents, they are deemed to conform.

The parameters used encompass physical properties that can be assessed relatively quickly and can provide a means of rapidly screening samples. Common tests include relative density, refractive index, optical rotation, miscibility in ethanol, and acid value, which are discussed in some detail below. Other commonly used standard methods are depicted in Table 2.

These physical and chemical tests can be performed in many general analytical chemistry laboratories. However, for the reports to be internationally recognized, the specified standards methods should be added to the scope of accreditation of the laboratories concerned (see comments on accreditation in section 9.1.2).

The analysis of essential oils relies extensively on chromatographic techniques to break the substances down into the constituent aromatic compounds, to identify them, and to measure the relative concentrations of each.

While several alternative methods are commonly used and have been published as standard methods, gas chromatography (GC) with a narrow-bore capillary column and a flame ionization detector (FID), is listed by ISO TC 54 as a reference method, which can be used for most preparations. The GC profile or chromatogram with the key peaks, their relative retention times (retention index) and the relative concentrations (determined by integration or measuring the areas under the peaks), is used as a fingerprint to uniquely identify the substance.

Chromatography can be used to not only identify molecules but, through advanced techniques, can isolate enantiomers of molecules in samples to, for example, differentiate between oils isolated from plants grown in certain regions. This technique is also used to detect and identify residues and other substances present in trace amounts in samples, such as residues of pollens or pathogens, which can aid in the positive affirmation of quality or the identification of substances of concern

Chromatography is a relatively common technique, practiced in many chemical testing laboratories. However, the analysis of essential oils poses challenges in terms of the matrices of the samples being tested and the need to adjust the chromatographic conditions to achieve clear separation of the indicator peaks. This may require the need to acquire specific columns for some analyses, and to validate these before use.

Users of test reports should therefore ensure that the scopes of accreditation include the techniques and sample matrices.

The analysis of essential oils relies extensively on chromato-graphic techniques to break the substances down into the constituent aromatic compounds, to identify them, and to measure the relative concentrations of each.

# 7 Physical and chemical testing of essential oils

## 7.1 Relative density

- ISO 279, Essential oils – Determination of relative density at 20 °C – Reference method

The specific density (ρ) is a common physical test conducted in most analytical chemistry laboratories, making use of a calibrated pycnometer and balances. Density is a measure of mass per unit volume (g/cm$^3$). The measurement is conducted at 20 °C.

Because the specific density is affected by pressure and other environmental factors, the result is reported relative to the density of a standard substance which, unless otherwise stated, is water, which has a specific density of 1 at 4 °C. Relative density (RD) is dimensionless, and calculated as follows:

$$RD = \frac{\rho \text{ sample}}{\rho \text{ reference}}$$

The relative density can be used as one of the indicators of the purity or authenticity of the essential oil.

## 7.2 Refractive index

The refractive index of a substance is the extent to which the angle of light is altered as light passes through it. This quality is used, for example, in optics to focus or magnify light as it passes through lenses.

- ISO 280, Essential oils – Determination of refractive index

The refractive index of a substance is the extent to which the angle of light is altered as light passes through it. This quality is used, for example, in optics to focus or magnify light as it passes through lenses. The refractive index of solutions is related to a substance in solution and also to its concentration. In the food industry, for instance, hand-held refractometers are used to measure sugar concentrations and is a common process control step. Volatile oils are characterized by high refractive indices. The refractive index of volatile oils varies from 1,43 to 1,61 (the refractive index of pure water at 20 °C is 1,333). By referring to product standards of the genuine oil, it can be gleaned whether the specimen is of the desired concentration, and if it is genuine or adulterated.

## 7.3 Optical rotation

- ISO 592, Essential oils – Determination of optical rotation

When polarized light passes through certain substances in solution, they have the ability to change the phase angle of the light passing through the solution essentially because the molecules are asymmetrical. Organic compounds that are chemically identical can differ structurally and become mirror images of one another when viewed three-dimensionally, they also then either rotate light to the left (known as laevorotatory, written with the prefix *L*), or to the right (known as dextrorotatory, written with the prefix *D*). These substances are said to be chiral in that they have mirror images of one another, which are known as enantiomers.

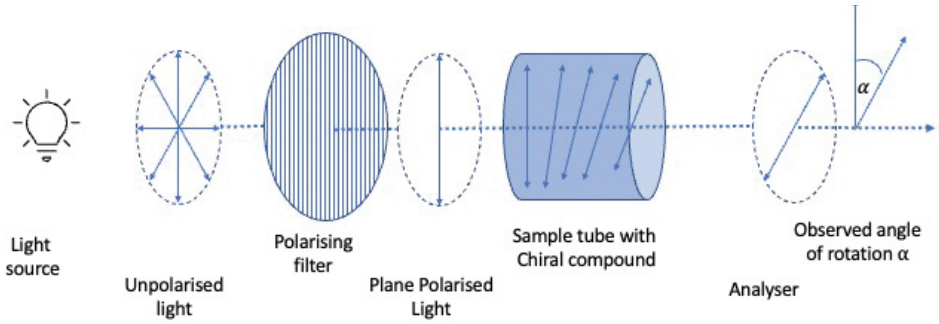


*Figure 1: Diagram illustrating optical rotation through a typical sugar solution*

Most of the aromatic compounds in vegetable and essential oils are optically active. The specific rotation is often a valuable diagnostic property, which is related to the chemical constitution of the oil or its constituents. The optical rotation and specific rotation give an indication whether the oil is genuine or adulterated. In some cases, it may give an indication of the variety of the oil (levo or dextro), e.g. French oil of turpentine is levo, while American turpentine is dextro. It may further indicate whether the substance is natural or synthetic, e.g. natural menthol is levo-rotatory, while the synthetic version may be levo or racemic (a mixture of both that does not show any rotation). Also, natural camphor is dextro, while synthetic camphor is levo or racemic.

## 7.4. Miscibility in ethanol

- ISO 875, Essential oils – Evaluation of miscibility in ethanol

Volatile oils as a rule are immiscible with water, but they are sufficiently soluble (very sparingly) to impart their odour and taste to water, and the olfactory aromatic waters are dependent on this slight solubility. They are fairly soluble in dilute alcohol. The difference in the solubility of the volatile oil in alcohol of different strengths can be used to detect common adulterants of volatile oils. For example, fixed oil or petroleum ether, when added to the volatile oil, lessens its solubility in alcohol. To carry out this test, 1 ml of the oil is introduced into a millilitre-graduated measuring cylinder, then alcohol of known strengths (95, 90, 80, 70, 60, 50%), added, until complete solution is effected (at a known temperature).

Most of the aromatic compounds in vegetable and essential oils are optically active. The specific rotation is often a valuable diagnostic property, which is related to the chemical constitution of the oil or its constituents.

## 7.5 Acid value

- ISO 1242, Essential oils – Determination of acid value

The acid value is a common physical test, defined as the number of milligrams of potassium hydroxide required to neutralize the free acids in 1 g of the oil, which indicates the amount of free acids present in the oil. High acid values arise in rancid oils.

Other standard test methods in the field of essential oils published by ISO include:

| | |
|---|---|
| **ISO 709** | Essential oils – Determination of ester value |
| **ISO 1041** | Essential oils – Determination of freezing point |
| **ISO 1271** | Essential oils – Determination of carbonyl value – Free hydroxylamine method |
| **ISO 1272** | Essential oils – Determination of content of phenols |
| **ISO 1279** | Essential oils – Determination of carbonyl value – Potentiometric methods using hydroxylammonium chloride |
| **ISO 3794** | Essential oils (containing tertiary alcohols) – Estimation of free alcohols content by determination of ester value after acetylation |
| **ISO 4715** | Essential oils – Quantitative evaluation of residue on evaporation |
| **ISO 7660** | Essential oils – Determination of ester value of oils containing difficult-to-saponify esters |
| **ISO 11021** | Essential oils – Determination of water content – Karl Fischer method |
| **ISO 18321** | Essential oils – Determination of peroxide value |
| **ISO 4735** | Oils of citrus – Determination of CD value by ultraviolet spectrometric analysis |

*Table 2: List of widely used standard physical and chemical test methods commonly used for essential oils*

# 7 Physical and chemical testing of essential oils

## 7.6 Chromatographic profiles of essential oils

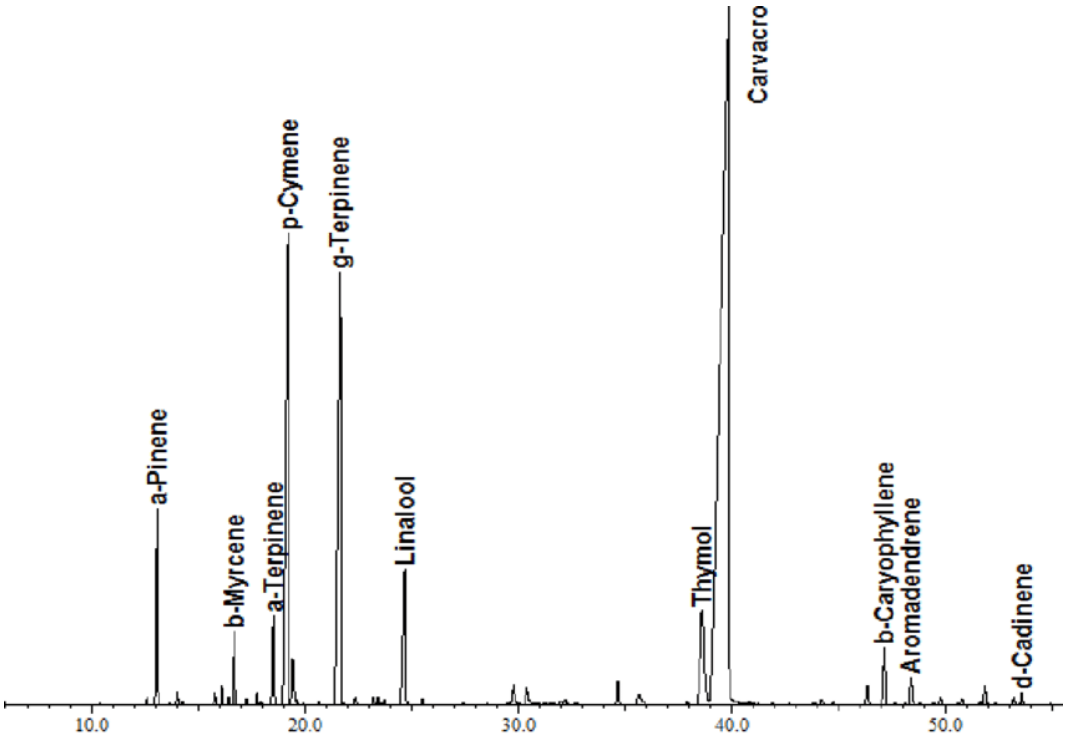


*Figure 2: Typical example of a gas chromatogram of an essential oil – the example provided is for thyme oil*
*Source: August 2020 Scientia Pharmaceutica 88(3):33.*

- **International Standard ISO 11024** consists of the following parts, under the general title Essential oils – General guidance on chromatographic profiles:
  - Part 1: Preparation of chromatographic profiles for presentation in standards
  - Part 2: Utilization of chromatographic profiles of samples of essential oils
- **ISO 7359 – Essential oils** – Analysis by gas chromatography on packed columns – General method
- **ISO 7609 – Essential oils** – Analysis by gas chromatography on capillary columns – General method
- **ISO 17494 –** Aromatic extracts, flavouring and perfuming compounds – Determination of ethanol content – Gas chromatographic method on packed and capillary columns
- **ISO 18054 –** Oils of orris rhizome (Iris pallida Lam. or Iris germanica L.) – Determination of irone content – Method using gas chromatography on a capillary column
- **ISO 8432 –** Essential oils – Analysis by high performance liquid chromatography – General method.

Chromatography is used extensively as a technique to isolate and identify the different aromatic compounds in the oil.

The ISO standards above provide guidance on the preparation of a standard mixture of aromatic compounds and the acceptable baseline separation which should be achieved to characterize an essential oil.

For general identification and characterization of the oils, detection is done using a flame ionizing detector (FID).

The retention indices of peaks can be used to identify the components of the oils.

The technique can be used to identify the representative components in a sample of an essential oil – that are defined as all the components of the oil that are present in the sample, which are evident as peaks on the chromatogram. Standard methods such as ISO 11024 mentioned above, provide guidance on the formulation of a reference standard against which to compare a sample to be able to correctly identify the peaks on the chromatogram associated with the components of the essential oil. These certified reference materials (CRMs) are commercially available from several suppliers, such as the US Pharmacopeia (USP).

The chromatogram of an essential oil can be used to identify the characteristic components or the relative concentration (or even absence) of different components in the oils, which can be used to identify the oil or to ascertain its purity. For example: *Guaia 9,6 diene is present in traces of Africa geranium and present in higher concentrations in the bourbon geranium, and 10-Epi-gamma-eudesmol is absent in bourbon geranium but present in the Africa geranium* (Source: ISO 11024).

The chromatogram of an essential oil can be used to identify the characteristic components or the relative concentration (or even absence) of different components in the oils, which can be used to identify the oil or to ascertain its purity.

# 8 Physical and chemical testing of vegetable oils

The testing of vegetable oils is well established, and covers tests to determine the chemical structure of the oils and to detect evidence of spoilage or denaturing of the oils. Several standard test methods have been published by international standards organizations, which have been used in the industry for many years.

Standards and certifications bodies, such as the American Organisation for Agricultural Chemists (AOAC), the American Oil Chemists Society (AOCS), the American Standards and Test Methods (ASTM) and ISO, publish reference test methods for these products. As with essential oils, ISO has a committee dedicated to the animal and vegetable fats and oils, falling under the technical committee for food products. This committee ISO TC 34/SC 11 *(Food products / Animal and vegetable fats and oils)* is active in this area, and has published the methods referred to below.

Because the production of fats and oils is a well-established industrial process, innovations related to these test methods continue, resulting in several alternative methods having become available.

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Because the production of fats and oils is a well-established industrial process, innovations related to these test methods continue, resulting in several alternative methods having become available. Some of the reference methods are discussed below.

## 8.1 Iodine value (vegetable oils)

- ISO 3961:2018 Animal and vegetable fats and oils – Determination of iodine value

The iodine value of an oil is a standard that measures the degree to which the fatty acids contained in the oil or wax are unsaturated. It is measured by the amount of iodine (in grams), that is absorbed by 100 g of the substance.

## 8.2 Saponification value

- ISO 3657:2020 Animal and vegetable fats and oils – Determination of saponification value

The saponification value or number pertains to all fatty acids present in the sample. To determine the saponification value, the sample is completely saponified with an excess of alkali, which excess is then determined by titration (in mg KOH/g).

## 8.3 Peroxide value

- ISO 3960:2017 Animal and vegetable fats and oils – Determination of peroxide value – Iodometric (visual) endpoint determination

The double bonds found in fats and oils play a role in autoxidation. Oils with a high degree of unsaturation are most susceptible to autoxidation. The best test for autoxidation (oxidative rancidity) is the determination of the peroxide value.

## 8.4 Anisidine value

- ISO 6885:2016 Animal and vegetable fats and oils – Determination of anisidine value

The chemical analysis method for the p-anisidine value determines the amount of aldehydes in vegetable oils and fats by the reaction of these compounds with the p-anisidine. Like the peroxide test above, this is an indication of the quality of the oil as the anisidine reaction highlights the concentration of the quantity of aldehydes and ketones, giving the dimension of the secondary oxidation of the fat matrices – all of which are indicators of possible spoilage.

## 8.5 Unsaponifiable material fatty acids

- ISO 3596:2000 Animal and vegetable fats and oils – Determination of unsaponifiable matter – Method using diethyl ether extraction

The unsaponifiable matter is that fraction of oil and fat that is not saponified with caustic alkali, but is soluble in non-polar solvents. The unsaponifiable matter in oil or fat consists of hydrocarbons, higher alcohols, oil-soluble vitamins and sterols which are not soluble in water after esterification. Most oils and fats of normal purity contain less than 2% unsaponifiable matter. Higher values indicate the possibility of adulteration with mineral oil. Adulteration of oils and fats with paraffin hydrocarbons will appear in the unsaponifiable matter.

## 8.6 Insoluble impurities

- ISO 663:2017 Animal and vegetable fats and oils – Determination of insoluble impurities content

This is a measure of the presence of dirt, minerals, resins, oxidized fatty acids, alkaline soaps of palmitic and stearic acids and proteins that are suspended in the oil.

It is determined by dissolving some oil in hexane or petroleum ether and filtering out the impurities, and is expressed as a percentage (%) of the total.

Most oils and fats of normal purity contain less than 2% unsaponifiable matter. Higher values indicate the possibility of adulteration with mineral oil.

## 8.7 Moisture

- ISO 8534:2017 Animal and vegetable fats and oils – Determination of water content – Karl Fischer method (pyridine free)

The moisture content of the oil is determined by Karl Fischer titration. It is an indicator of the quality of the oil and an important consideration for further use in product formulations.

## 8.8 Free fatty acid (FFA) and acid value

- ISO 660:2020 Animal and vegetable fats and oils – Determination of acid value and acidity

The acid value is defined as the amount of FFA present in the oil requiring a computed mass of potassium hydroxide (KOH) to neutralize it. This value is used to determine the amount of base catalyst to neutralize the acidity of a gram of raw material. The acid value *(AV)* is reported in *mg* of KOH per *g* of sample and is determined by titration of with a KOH solution of known concentration.

# 9 Some information on laboratory competence

The claims regarding the characterization, performance, quality and purity of oils and other ingredients are based on assessments carried out in laboratories. As indicated above, a wide range of tests may be conducted, covering aspects such as chemical, physical, and organoleptic properties, and more. Some of the tests may be regarded as being routine and can be performed by several laboratories, while others are quite specialized and may not be as readily available.

Suppliers, buyers and regulators all require testing for various stages of production, development, registration, packaging, transport, storage, labelling and selling of the product. Independent third-party testing is often desirable in these cases as, if the laboratory has no obvious financial interest in the outcome of the result, the results are more likely to be trusted.

As with the test results themselves, the issuing laboratories need to be competent to perform those tests and provide the reports based on the assessments.

The basis used by laboratories to demonstrate their competence is accreditation, which is, in short, a third-party assessment of the equipment, personnel, processes and systems in the laboratory related to the ability to perform tests in a defined technical area. When dealing with the accreditation of laboratories, two approaches are commonly used. These are the *Principles of Good Laboratory Practice* (GLP), published by the OECD, and the ISO/IEC 17025 (2017) *General Requirements for the competence of testing and calibration laboratories*, published by the International Organization for Standardization (ISO) and the International Electrotechnical Commission (IEC).

The OECD developed a set of internationally harmonized good laboratory practices which were published in 1981 as the *OECD Principles of Good Laboratory Practice* (OECD GLP).

Both processes serve to provide confidence that the recognized bodies are competent and that the results they provide can be trusted. The two approaches differ in terms of how they recognise competence and how that recognition is normally applied. The two approaches are briefly explained below.

## 9.1 Introduction and history

### 9.1.1 Good laboratory practice (GLP)

Good laboratory practices were developed in the 1970s in support of applications for the regulatory registration/approval of chemicals. They were developed by governments as a regulatory control mechanism to ensure future safety studies would be of acceptable quality and integrity.

In addition, good laboratory practices were developed to apply to testing facilities that conducted non-clinical health and environmental safety studies for submission to a government regulatory agency in support of a regulated product.

The OECD developed a set of internationally harmonized good laboratory practices which were published in 1981 as the *OECD Principles of Good Laboratory Practice* (OECD GLP). The said principles are adhered to by facilities carrying out studies to be submitted to national regulatory authorities for the purpose of assessing the health and environmental safety of chemicals and chemical products (which may also be of natural or biological origin) and, in some circumstances, may be living organisms.

As a regulatory control mechanism, OECD GLP compliance has been written into law in many countries. OECD GLP originated from, and remains an integral part of, the regulatory sector.

### 9.1.2 Laboratory accreditation (ISO/IEC 17025)

ISO/IEC 17025, on the other hand, was developed by the testing/calibration laboratory and laboratory accreditation communities. Originally published as ISO Guide 25 in 1978, its origins were in the laboratory accreditation community, who prepared a mutually agreed upon set of criteria that a laboratory should fulfil in order to demonstrate its technical competence.

ISO/IEC 17025 was initially published in 1999, with a minor revision in 2005, and a new edition was published in 2017.

ISO/IEC 17025 was, as with all standards published by the International Organization for Standardization (ISO), written by nominated experts from national standards bodies who were members of ISO. The standard was agreed to and published after an extensive international review and comment process.

ISO/IEC 17025 can be implemented by laboratories involved in all areas of testing and calibration, including non-clinical testing, no matter what their size or complexity.

While laboratory accreditation has its origins in the private, non-regulated sector, governments around the world are increasingly specifying international standards, such as ISO/IEC 17025, as a tool to meet their regulatory objectives across a wide range of fields.

## 9.2 The application

The OECD GLP is a set of principles applied to the conduct of testing studies that are intended for submission to the appropriate regulatory authorities in support of the registration, licensing or regulation of chemical and related products. The OECD GLP is neither intended nor required for non-regulated testing.

For the sort of testing that is regulated and is required to be conducted under OECD GLP, the testing is often scientifically multi-disciplinary, and individual tests may be conducted over several months. For example, traditionally, OECD GLP has been applied to toxicological testing. Long-term toxicology studies may run for several months and involve many scientific disciplines, such as analytical and bio-analytical chemistry, clinical pathology testing, histopathology, physical testing and the like. Each study will generally involve a new chemical test. The individual assays within each study will therefore vary from study to study, and may never be used again once the suite of testing has been completed.

For the sort of testing that is regulated and is required to be conducted under OECD GLP, the testing is often scientifically multi-disciplinary, and individual tests may be conducted over several months.

In addition, these studies may be conducted outside a traditional laboratory setting and the OECD GLP is therefore, of necessity, quite general in its requirements, and takes the form of a set of principles. This allows them to accommodate the wide variety of studies undertaken, the scientific disciplines involved, and the variability within studies for the different chemicals under test.

Most importantly, the focus of the OECD GLP is on the individual study. A "study" being a discrete package of work passing through the test facility that is conducted in accordance with a "study plan" that culminates in a single "study report," and submitted to the regulator.

ISO/IEC 17025 is a technical competence and management system standard developed specifically for testing and calibration laboratories. ISO/IEC 17025 can therefore be applied to a broad range of laboratories, including non-clinical laboratories. This includes laboratories that conduct the assays on a regular basis according to defined methodology, where the type of samples tested and the test methods employed vary little from day to day.

The focus of ISO/IEC 17025 is on the competence of and systems available within the laboratory that support and provide critical input into how the laboratory conducts its testing services, both at the technical and management levels.

There are certain types of regulated non-clinical health and environmental safety testing that could be effectively conducted under an ISO/IEC 17025 system, for example, physical/chemical tests to determine these properties for a regulated chemical product.

For the majority of regulated non-clinical health and environmental safety tests, compliance with the OECD GLP is best suited. This is due to issues such as the variability inherent in such studies (arising from living test systems), the scientific multi-disciplinary nature of the studies, the multi-site nature of such

# 9 Some information on laboratory competence

studies, and the differences in the chemical product under test in each study. The OECD GLP has been specifically designed to accommodate the management of such variability.

The requirements of the OECD GLP and ISO/IEC 17025 differ, for good reason. For example, the OECD GLP has very specific requirements with regard to quality assurance activities and for the Study Director, who has overall responsibility for all phases of the study and who holds a crucial role in OECD GLP. ISO/IEC 17025, on the other hand, includes requirements that are not covered by the OECD GLP. Laboratories that are involved in the non-regulated arena may need to focus on additional elements, such as customer requirements, ongoing quality improvement, and technical aspects such as internal quality control and external proficiency testing.

The OECD GLP is used as a regulatory control mechanism to assure the quality and integrity of non-clinical health and environmental safety studies regulated under law.

Laboratory accreditation provides a mechanism to establish the technical competence of laboratories to perform specific tests. A "scope of accreditation" describes the laboratory's activities for which competence has been determined and awarded. The scope may be quite detailed or very broad, depending on the nature of the laboratory and the services it provides. To maintain accreditation, laboratories are re-evaluated periodically by the accreditation body to ensure their continued compliance with requirements, and to ensure that their standard of operation is maintained. The laboratory may also be required to participate in relevant proficiency testing programs between reassessments as a further demonstration of technical competence.

For OECD GLP studies, the responsibility for evaluating the technical validity of a study (study design) and validity of the conclusions drawn from the study results, lies with the regulatory reviewer. However, this evaluation can only be effective if the study data can be relied upon, the quality and scientific integrity of the data can be demonstrated, and the conduct of the study reconstructed. An OECD GLP quality system is designed specifically to meet this need. The focus of the OECD GLP quality system is on the administration and management of the conduct of the study, rather than the science of the study being undertaken.

## 9.3 Summary

While the OECD GLP and ISO/IEC 17025's general requirements for the competence of testing and calibration laboratories both set out requirements for quality management systems under which testing is to be conducted, they are, as a result of their evolution and history, documents with different purposes. It is therefore impractical, and in many cases would be inappropriate, to apply one set of requirements with the intention of meeting the purposes of the other.

The OECD GLP is used as a regulatory control mechanism to assure the quality and integrity of non-clinical health and environmental safety studies regulated under law. Such testing, for the most part, is complex and variable, and the OECD GLP is specifically designed, as a set of principles, to be applied to individual studies to accommodate the complexity and variability of such studies.

ISO/IEC 17025 is an international standard intended to be applied to laboratory facilities conducting testing according to established or specifically developed methodologies. The focus of the standard is on the on-going operation and management of the laboratory itself, and on the capacity of the laboratory to produce consistent and reliable results that are scientifically valid. ISO/IEC 17025 can, in theory, be applied to any testing laboratory in any scientific discipline, including those performing non-clinical testing.

GLP Compliance Monitoring is a regulatory inspection with the intent of verifying that individual non-clinical health and environmental studies, submitted to receiving authorities for the purpose of registration/approval of chemical products, meet the requirements of the law (i.e. that the study has been conducted in accordance with the national GLP regulations). The focus of such inspections is on the studies conducted, and audits of individual studies make up a significant component of the

inspection. The main "customer" of GLP compliance monitoring inspections is the authorities to which the studies have been submitted.

As the application of OECD GLP is harmonized across OECD countries, governments can accept data from other countries with the assurance that this data will be valid and of acceptable quality. This is the basis of the Mutual Acceptance of Data (MAD) agreement, which is an integral part of the OECD GLP and requires regulators, whose governments adhere to MAD, to accept data from OECD GLP studies that have been conducted by facilities that had been inspected by the relevant national GLP compliance monitoring authority. The agreement is also open to non-OECD countries that adhere to MAD.

Laboratory accreditation provides formal third-party recognition to competent laboratories. A laboratory must be formally accredited before it can issue reports under the terms of its accreditation scope. This enables customers to identify and select reliable testing services able to meet their needs.

Laboratory accreditation is highly regarded nationally, regionally and internationally. It is a reliable indicator of technical competence, and many industries routinely specify laboratory accreditation for suppliers of testing services.

There are multilateral arrangements between the various national accreditation bodies for recognition of each other's accreditations (e.g. the International Laboratory Accreditation Cooperation Mutual Recognition Arrangement[6] (ILAC MRA)). The accreditation bodies that are signatories to the ILAC MRA have been peer evaluated in accordance with the requirements of ISO/IEC 17011:2017[7] *(Requirements for accreditation bodies accrediting conformity assessment bodies)* to demonstrate their competence. ILAC MRA signatories agree to accept the results from each other's accredited laboratories under the ILAC MRA. Hence, the results from laboratories accredited by the ILAC MRA signatories are recognized internationally.

It should be noted, however, that the decision to accept results from accredited laboratories remains with the end-user.

Laboratory accreditation is increasingly being used by governments to meet regulatory and trade objectives. It is not, however, applied to non-clinical health and environmental safety testing because ISO/IEC 17025 does not contain all of the requirements of the OEDC GLP's principles. Nevertheless, laboratory accreditation can make a valuable contribution within the GLP compliance structure.

Laboratory accreditation is highly regarded nationally, regionally and internationally. It is a reliable indicator of technical competence.

6 *https://ilac.org/ilac-mra-and-signatories/*

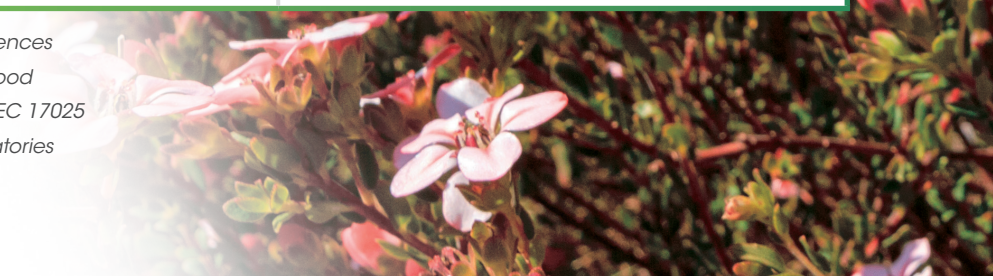
7 *ISO/IEC 17011:2017 Conformity assessment – Requirements for accreditation bodies accrediting conformity assessment bodies – www.iso.org*

# 9 Some information on laboratory competence

| OECD GLP | ISO/IEC 17025 |
|---|---|
| Assures the quality and integrity of non-clinical health and environmental safety studies regulated under law.<br><br>GLP was designed, as a set of principles, to be applied to individual studies to accommodate the complexity and variability of such studies. | International standard intended to be applied to laboratory facilities conducting testing according to established or specifically developed methodology.<br><br>Focuses on the operation and management of the laboratory itself, and on the capacity of the laboratory to produce consistent and reliable results that are scientifically valid.<br><br>ISO/IEC 17025 can be applied to any testing laboratory. |
| The main "customer" of GLP compliance monitoring inspections is the authorities to which the studies have been submitted.<br><br>Audits of individual studies make up a significant component of the inspection. | Laboratory accreditation provides formal third-party recognition to competent laboratories.<br><br>Accreditation process includes assessment of the competencies and systems used to conduct tests. |
| Governments can accept data from other countries – this is the basis of the Mutual Acceptance of Data (MAD) agreement, which is an integral part of the OECD GLP.<br><br>Regulators, whose governments adhere to MAD, are required to accept data from OECD GLP studies that have been conducted by facilities that have been inspected by the relevant national GLP compliance monitoring authority. | A laboratory must be formally accredited before it can issue reports under the terms of its accreditation scope.<br><br>Laboratory accreditation is a reliable indicator of technical competence.<br><br>Many industries routinely specify laboratory accreditation for suppliers of testing services. |
| Laboratory accreditation can make a valuable contribution within the GLP compliance structure. | The decision to accept results from accredited laboratories remains with the end user.<br><br>Laboratory accreditation is increasingly being used by governments to meet regulatory and trade objectives. It is not, however, applied to non-clinical health and environmental safety testing because ISO/IEC 17025 does not contain all of the requirements of the OEDC GLP principles. |

*Table 3: Summary of the differences between the application of good laboratory practice and ISO/IEC 17025 accreditation to testing laboratories*

Laboratory accreditation can make a valuable contribution within the GLP compliance structure.



# 10 Concluding remarks

Laboratory testing provides objective evidence that can support many aspects of the production and marketing of vegetable oils and essential oils. Testing these products offers some challenges to those conducting the analysis in terms of the instruments and other physical resources, and the competencies and experience in dealing with these specific samples. Laboratories that conduct all or some of the required analyses are not very common or evenly distributed across the country, which poses additional challenges to those wishing to explore opportunities to get product samples tested.

The tests that are required and the laboratories that should be considered to conduct those tests will differ depending on the purpose the results of those tests are intended for, so it is useful to understand and clearly articulate the testing needs at the start of the testing process.

Testing of products can be time consuming and expensive and, while there are several tests that can be performed, requesting the right tests at the right time from a laboratory that is appropriately accredited can ensure that the money is effectively spent, and subsequent (even more expensive) surprises, such as product rejection or the need to have it retested, can be avoided.

Laboratory testing provides objective evidence that can support many aspects of the production and marketing of vegetable oils and essential oils.

# Notes

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This publication has been sponsored by the project "Strengthening the quality of essential and vegetable oils exports from South Africa". The Global Quality and Standards Programme (GQSP) is a large-scale programme, which was designed to encourage systematic trade development along specific value chains by strengthening quality infrastructure institutions and service providers, enhancing the compliance capacity of private sector actors, particularly Small and Medium Enterprises (SMEs), and creating a culture of quality among all stakeholders. The GQSP is implemented by UNIDO and funded by Switzerland, through the Swiss State Secretariat for Economic Affairs (SECO).

The objective of the GQSP South Africa (GQSP-SA) project is to strengthen the quality and standards compliance capacity to facilitate market access for SMEs in the essential and vegetable oils value chain destined for food, health and cosmetic markets.



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