

How ISO4730:2004 and AS 2782-2009 Standards Help Identify Fraudulent Tea Tree Oil

Pure Australian tea tree oil (TTO) steam distilled from *Melaleuca alternifolia* should always conform to the International Standard ISO 4730 (2004) and the identical Australian Standard AS 2782-2009 ("Oil of *Melaleuca*, *Terpinen-4-ol* type"), which specifies the levels of 15 of the 113+ components as well as several physical parameters of pure Australian TTO. When a sample of TTO is sent to a laboratory for analysis a minimum of 15 ml is usually required.

The specifications are listed in the table below:

Component	ISO 4730:2004 Range	
	Minimum %	Maximum %
α -Pinene	1.00	6.00
Sabinene	trace	3.50
α -Terpinene	5.00	13.00
Limonene	0.50	1.50
p-Cymene	0.50	8.00
1,8-Cineole	trace	15.00
γ -Terpinene	10.00	28.00
Terpinolene	1.50	5.00
Terpinen-4-ol	30.00	48.00
α -Terpineol	1.50	8.00
Aromadendrene	trace	3.00
Ledene	trace	3.00
δ -Cadinene	trace	3.00
Globulol	trace	1.00
Viridiflorol	trace	1.00
Physical Properties	ISO 4730:2004 Range	
	Minimum %	Maximum %
Appearance	Clear, mobile liquid without visible water	
Colour	Colourless to pale yellow	
Odour	Characteristic, Myrtistic	
Relative Density (20 °C)	0.885	0.906
Refractive Index (20 °C)	1.475	1.482
Optical rotation (20 °C)	+ 5.00 °	+ 15.00 °
Miscibility in 85% (v/v) ethanol (20 °C)	Less than 2 volumes	

The TTO Standard provides a range for all objective parameters to allow for the variation that occurs in natural products such as pure Australian TTO. This natural variability is usually caused by slight differences in growing conditions from year to year, minor genetic variation from plantation to plantation and differing distillation techniques used.

Note: Minor variations can also occur with identical samples. This is usually due to differing conditions (temperature, humidity, atmospheric pressure etc) when an analysis is done.

The Parameters Explained

Component Range

Pure TTO contains 113+ compounds which can be identified and quantified using a gas chromatograph (GC). It is impractical to base a standard on all 113+, so standards committees look very carefully at the GC profile for a substance and choose a limited number of components based on both their importance and uniqueness. This is similar to a fingerprint search: is not possible to compare the entire print, so reference points are used. If the sample does not contain all 15 substances in the ranges given then it cannot be sold or used as pure TTO. The absence of a compound is just as telling as having too much, especially the 7 compounds where the minimum % is 'trace'.

Physical Properties

Because cheating (extending, adulterating etc) can occur, standards committees also provide a list of physical properties to help an analyst to determine the likelihood of a sample being pure TTO. Some of these are advisory while others are compulsory. They are a mix of subjective and objective properties.

Note: On very rare occasions, pure Australian TTO steam distilled from Melaleuca alternifolia doesn't fully conform to the standard (eg high p-cymene or low terpinene-4-ol).

Subjective properties

The appearance, colour and odour are subjective (are not measured precisely). The Standard describes these so an analyst can state that it either conforms or fails.

Objective properties

These are measured using calibrated precision instruments. The results are compared to the Standard to either conform or fail.

Relative Density

Relative density (RD) is the ratio of the density (mass of a unit volume) of a substance to the density of a given reference material, usually water. If the RD = 1 then it is probably pure water. If the RD is less than 1.00 it is less dense than water and will float. A good example is ice which has an RD of 0.91.

For pure TTO the RD at 20 °C must be between 0.885 and 0.906.

Refractive Index

Refractive index (RI) is the measurement of how hard it is for light to travel through a medium. The higher the number the harder it is. It is compared to air with an RI of 1.00. Some examples of the RI for liquids at 20 °C:

Water	1.333
Ethanol	1.360
Benzene	1.501.

For pure TTO the RI at 20 °C must be between 1.475 and 1.482.

Optical Rotation

When plane-polarized light is passed through a sample of known chiral content, the plane of the polarised light is rotated by a very specific and measurable quantity. This is known as the optical rotation (OR) of a substance. This technique is also used to measure the purity and concentration of a number of familiar chemicals:

Sucrose	+66.47°
Cholesterol	-31.5°
Camphor	+44.26°
Penicillin V	+223°

For pure TTO the OR at 20 °C must be between +5.00° and +15.00°.

Miscibility in 85% (v/v) ethanol

It should be possible to completely dissolve a measured volume of pure TTO in less than twice that volume of 85% ethanol to obtain a clear solution.

An Example of a conforming and a non-conforming sample

The following table lists the results of two samples recently analysed by the DPI at Wollongbar, one is pure Australian TTO that has been grown under strict quality assurance while the other has clear evidence of adulteration:

Component	ISO 4730:2004 Range		Pure Australian TTO		Adulterated "TTO"	
	Minimum %	Maximum %	Result	Outcome	Result	Outcome
α-Pinene	1.00	6.00	2.40	conforms	3.10	conforms
Sabinene	trace	3.50	0.20	conforms	0.10	conforms
α-Terpinene	5.00	13.00	9.50	conforms	0.20	fail
Limonene	0.50	1.50	0.80	conforms	3.40	fail
p-Cymene	0.50	8.00	2.00	conforms	7.30	conforms
1,8-Cineole	trace	15.00	2.10	conforms	4.70	conforms

Component	ISO 4730:2004 Range		Pure Australian TTO		Adulterated "TTO"	
	Minimum %	Maximum %	Result	Outcome	Result	Outcome
γ-Terpinene	10.00	28.00	20.80	conforms	23.40	conforms
Terpinolene	1.50	5.00	3.40	conforms	3.50	conforms
Terpinen-4-ol	30.00	48.00	42.80	conforms	39.10	conforms
α-Terpineol	1.50	8.00	2.90	conforms	6.10	conforms
Aromadendrene	trace	3.00	1.30	conforms	0.00	fail
Ledene	trace	3.00	0.90	conforms	0.10	conforms
δ-Cadinene	trace	3.00	1.00	conforms	0.00	fail
Globulol	trace	1.00	0.20	conforms	0.00	fail
Viridiflorol	trace	1.00	0.10	conforms	0.00	fail
Physical Properties	ISO 4730:2004 Range		Pure Australian TTO		Adulterated TTO	
	Minimum %	Maximum %	Result	Outcome	Result	Outcome
Appearance	Clear, mobile liquid		conforms	conforms	conforms	conforms
Colour	Colourless to pale yellow		conforms	conforms	conforms	conforms
Odour	Characteristic, Myrtistic		conforms	conforms	conforms	conforms
Relative Density (20 °C)	0.885	0.0906	0.896	conforms	0.897	conforms
Refractive Index (20 °C)	1.475	1.482	1.477	conforms	1.477	conforms
Optical rotation (20 °C)	+ 5.00 °	+ 15.00 °	+ 10.00 °	conforms	- 9.9°	fail
Miscibility in 85% (v/v) ethanol (20 °C)	Less than 2 volumes		0.60	conforms	0.50	conforms

Note that the pure Australian TTO conformed to all of the parameters in the standard and can therefore be sold as such. However the adulterated sample failed 7 of the total 22 parameters.

It is possible and maybe even likely that the product was originally an essential oil of some sort but it would have been of very poor quality (eg low terpinene-4-ol and high p-cymene). In an attempt to make the product conform, the perpetrator would have added synthetic terpinene-4-ol (often made from sabinene derived from pine oil), aiming for around 40%: what many buyers are after. Synthetic terpinen-4-ol has a different optical rotation to that found naturally in TTO so they would then also add synthetic limonene to balance this out.

If you look at the results, they got the terpinene-4-ol level spot on so it conforms; but they weren't very good at the rest:

1. The α-terpinene level is too low because too much synthetic terpinene-4-ol was needed to get close to the desired 40% level.
2. The limonene level is too high because they needed to add it to the product to balance the optical rotation.
3. Have a look at the p-cymene level: just inside the specification. This must have been really high before it was adulterated indicating it was probably very oxidised due to age, poor storage or both.
4. Aromadendrene, δ-cadinene, globulol and viridiflorol levels are below the limit of detection. This is precisely why these minor components are in the Standard: they are always in a pure, natural sample of TTO; if they are not present in at least a trace amount it is a signal that something is wrong.
5. The optical rotation is minus (-) 9.9°. Despite overdoing the limonene they still didn't get the optical rotation right. This is the equivalent of offering TTO with zero terpinen-4-ol and hoping no one will notice.

Conclusion

Adulterated material such as this is described and offered as 'tea tree oil' to world markets on a daily basis. It is not TTO, rather a mix of unknown chemicals from unidentified sources that have been thrown together with a single objective: profit. *No testing for safety and efficacy has ever been done on any of these concoctions.*

By claiming this fabrication as TTO the manufacturers rely on the years of research, good will and excellent reputation for safety and efficacy that pure Australian TTO (which has been steam distilled from *Melaleuca alternifolia*) enjoys.

Would you want to use this product?