Hyperici herba

DEFINITION
St. John’s wort consists of the whole or cut, dried flowering tops of Hypericum perforatum L., harvested during flowering time. It contains not less than 0.08 per cent of total hypericins expressed as hypericin (C_{30}H_{16}O_{8}; M_r 504.4), calculated with reference to the dried drug.

CHARACTERS
It has the macroscopic and microscopic characters described under identification tests A and B.

IDENTIFICATION
A. The branched and bare stem shows 2 more-or-less prominent longitudinal ridges. The leaves are opposite, sessile, extipulate, oblong-oval and 15 mm to 30 mm long; present on the leaf margins are glands which appear as black dots and over all the surface of the leaves many small, strongly translucent excretory glands which are visible in transmitted light. The flowers are regular and form corymbose clusters at the apex of the stem. They have 5 green, acute sepals, with black secrétoire glands on the margins; 5 orange-yellow petals, also with black secrétoire glands on the margins; 3 staminal blades, each divided into many orange-yellow stamens and 3 carpels surmounted by red styles.

B. Reduce to a powder (355). The powder is greenish-yellow. Examine under a microscope using chloral hydrate solution R. The powder shows the following diagnostic characters: fragments of polygonal cells of the epidermis with thickened and beaded walls and paracytic or anomocytic stomata (2.8.3); fragments of the leaf and sepal with large oil glands and red pigment cells; thin-walled, elongated cells of the petal epidermis with straight or wavy anticlinal walls; tracheids and tracheidal vessels with pitted walls and groups of thick-walled fibres; fragments of rectangular, lignified and pitted parenchyma; fibrous layer of the anther and elongated, thin-walled cells of the filament with a striated cuticle; numerous pollen grains with 3 pores and a smooth exine, occur singly or in dense groups, and calcium oxalate cluster crystals.

C. Examine by thin-layer chromatography (2.2.27), using a TLC silica gel plate R.

**Test solution.** Stir 0.5 g of the powdered drug (500) in 10 ml of methanol R in a water-bath at 60 °C for 10 min and filter.

**Reference solution.** Dissolve 5 mg of rutin R and 5 mg of hyperoside R in methanol R and dilute to 5 ml with the same solvent.

Apply to the plate as 10 mm bands 10 µl of the test solution and 5 µl of the reference solution. Develop over a path of 30 cm using a mixture of 6 volumes of anhydrous formic acid R, 9 volumes of water R and 90 volumes of ethyl acetate R. Allow the plate to dry at 100-105 °C for 10 min. Spray the plate with a 10 g/l solution of diphenylboric acid aminoethyl ester R in methanol R and then with a 50 g/l solution of macrogol 400 R in methanol R. Examine the plate after about 30 min in ultraviolet light at 365 nm. The chromatogram obtained with the reference solution shows in the lower third the zone due to rutin and above it the zone due to hyperoside, both with yellow-orange fluorescence. The chromatogram obtained with the test solution shows in the lower third the reddish-orange fluorescent zones of rutin and hyperoside and in the lower part of the upper third the zone of pseudohypericin and above it the zone of hypericin, both with red fluorescence. Other yellow or blue fluorescent zones are visible.

**TESTS**

**Foreign matter (2.8.2).** Not more than 3 per cent of stems with a diameter greater than 5 mm, and not more than 2 per cent of other foreign matter.

**Loss on drying (2.2.32).** Not more than 10.0 per cent, determined on 1.000 g of the powdered drug (500) by drying in an oven at 100-105 °C for 2 h.

**Total ash (2.4.16).** Not more than 7.0 per cent.

**ASSAY**

**Test solution.** In a 100 ml round-bottomed flask, introduce 0.800 g of the powdered drug (500), 60 ml of a mixture of 20 volumes of water R and 80 volumes of tetrahydrofuran R and a magnetic stirrer. Boil the mixture in a water-bath at 70 °C under a reflux condenser for 30 min. Centrifugé (2 min at 700 g) and decant the supernatant into a 250 ml flask. Take up the residue with 60 ml of a mixture of 20 volumes of water R and 80 volumes of tetrahydrofuran R. Heat again under a reflux condenser for 30 min. Centrifugé (2 min at 700 g) and decant the supernatant. Combine the extracts and evaporate to dryness. Take up the residue with 15 ml of methanol R with the help of ultrasound and transfer to a 25 ml measuring flask. Rinse the 250 ml flask with methanol R and dilute to 25.0 ml with the same solvent. Centrifugé again, filter 10 ml through a syringe filter (0.2 µm). Discard the first 2 millilitres of the filtrate. Introduce 5.0 ml of the filtrate into a measuring flask and dilute to 25.0 ml with methanol R.

**Compensation liquid.** Methanol R.

Measure the absorbance (2.2.25) of the test solution at 590 nm by comparison with the compensation liquid.
Calculate the percentage content of total hypericins, expressed as hypericin, from the expression:

\[ A \times 125 \]
\[ m \times 870 \]
i.e. taking the specific absorbance of hypericin to be 870.

\[ A = \text{absorbance at 590 nm,} \]
\[ m = \text{mass of the drug to be examined, in grams.} \]

**STANNOUS CHLORIDE DIHYDRATE**

Stannosi chloridum dihydricum

SnCl₂·2H₂O  \( M, 225.6 \)

**DEFINITION**

Stannous chloride dihydrate contains not less than 98.0 per cent and not more than the equivalent of 101.0 per cent of SnCl₂·2H₂O.

**CHARACTERS**

A white, crystalline powder or colourless crystals, efflorescent in air, freely soluble in water (the solution becomes cloudy after standing or on dilution), freely soluble in alcohol. It dissolves in dilute hydrochloric acid.

**IDENTIFICATION**

A. To 1 ml of solution S1 (see Tests) add a mixture of 5 ml of dilute hydrochloric acid R and 0.05 ml of mercuric chloride solution R. A blackish-grey precipitate forms.

B. Dissolve 1.0 g in 3.0 ml of water R. Add 0.5 ml of dilute sodium hydroxide solution R to the cloudy solution; a yellowish flocculent precipitate is formed. Add 6.5 ml of water R. To 1.0 ml of the previously shaken suspension add 1.0 ml of strong sodium hydroxide solution R; the precipitate dissolves and the resulting solution is clear and colourless.

C. Dissolve 10 mg in 2 ml of dilute nitric acid R. The solution gives reaction (a) of chlorides (2.3.1).

**TESTS**

**Solution S1.** To 0.40 g add 1 ml of dilute hydrochloric acid R and dilute to 20 ml with distilled water R.

**Solution S2.** Dissolve 1.0 g in dilute hydrochloric acid R and dilute to 30 ml with the same acid. Heat to boiling. Add 30 ml of thioacetamide solution R and boil for 15 min (solution A). Use 5 ml, filter and heat the filtrate to boiling. Add 5 ml of thioacetamide solution R and boil for 15 min. If a precipitate is formed, add the remainder of solution A (solution A') to the mixture. Add 10 ml of thioacetamide solution R and boil. Repeat the series of operations from “Use 5 ml,...” until a precipitate is no longer formed on addition of thioacetamide solution R to the filtrate obtained from the 5 ml of solution A (solution A', solution A'', respectively). If no precipitate is formed or if no more precipitate is formed combine the solution obtained with the remainder of solution A (solution A', solution A'', respectively), filter and wash the precipitate with 10 ml of water R. Heat the filtrate until the resulting vapour no longer turns a moistened piece of lead acetate paper R blackish-grey. Allow to cool and dilute to 50 ml with water R.

**Appearance of solution.** Dissolve 10.0 g in dilute hydrochloric acid R and dilute to 20 ml with the same acid. The solution is clear (2.2.1) and colourless (2.2.2, Method II).

**Substances not precipitated by thioacetamide.** Evaporate 25 ml of solution S2 to dryness and ignite at 600 °C. The residue weighs not more than 1 mg (0.2 per cent).

**Sulphates** (2.4.13). 15 ml of solution S1 complies with the limit test for sulphates (500 ppm).

**Iron** (2.4.9). Dilute 5 ml of solution S2 to 10 ml with water R. The solution complies with the limit test for iron (100 ppm).

**Heavy metals**. Dissolve 1.0 g in 2 ml of a mixture of 1 volume of nitric acid R and 3 volumes of hydrochloric acid R. Heat the solution on a water-bath until nitrous vapour is no longer evolved. Dissolve the residue in water R and dilute to 25 ml with the same solvent. To 5 ml of the solution add 3 ml of strong sodium hydroxide solution R and 2 ml of water R. Heat until a clear solution is obtained, then cool and add 0.5 ml of thioacetamide reagent R. After 2 min, any colour in the solution is not more intense than that of a mixture of 1.0 ml of lead standard solution (10 ppm Pb) R, 6 ml of water R, 3 ml of strong sodium hydroxide solution R and 0.5 ml of thioacetamide reagent R (50 ppm).

**ASSAY**

Dissolve 0.100 g in 1.5 ml of hydrochloric acid R1 and dilute to 50 ml with water R. Add 5 g of sodium potassium tartrate R, 20 g of sodium hydrogen carbonate R and 1 ml of starch solution R. Titrate immediately with 0.05 M iodine.

Carry out a blank titration.

1 ml of 0.05 M iodine is equivalent to 11.28 mg of SnCl₂·2H₂O.

**STORAGE**

Store in an airtight container.

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**STANOZOLOL**

Stanozololum

\( \text{C}_{31}\text{H}_{42}\text{N}_{2}\text{O} \)  \( M, 328.5 \)

**DEFINITION**

Stanozolol contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 17-methyl-2'H,5α-androst-2-eno[3,2-c]pyrazol-1β-ol, calculated with reference to the dried substance.

**CHARACTERS**

A white or almost white crystalline powder, hygroscopic, practically insoluble in water, soluble in dimethylformamide, slightly soluble in alcohol, very slightly soluble in methylene chloride.

It shows polymorphism.

**IDENTIFICATION**

A. Examine by infrared spectrophotometry (2.2.24), comparing with the spectrum obtained with stanozolol CRS. If the spectra obtained in the solid
**TANNIC ACID**

**Tanninum**

**DEFINITION**
Tannic acid is a mixture of esters of glucose with gallic acid and 3-galloylgallic acid.

**CHARACTERS**
A yellowish-white or slightly brown amorphous light powder or shiny plates, very soluble in water, freely soluble in alcohol and in glycerol (85 per cent), practically insoluble in methylene chloride.

**IDENTIFICATION**
A. Dilute 0.1 ml of solution S (see Tests) to 5 ml with water R. Add 0.1 ml of ferric chloride solution R1. A blackish-blue colour is produced which becomes green on the addition of 1 ml of dilute sulphuric acid R.
B. To 1 ml of solution S, add 3 ml of a 1 g/1 solution of gelatin R. The mixture becomes turbid and a flocculent precipitate is formed.
C. Dilute 0.1 ml of solution S to 5 ml with water R. Add 0.3 ml of barium hydroxide solution R. A greenish-blue precipitate is formed.

**TESTS**
**Solution S.** Dissolve 4.0 g in carbon dioxide-free water R and dilute to 20 ml with the same solvent.

**Appearance of solution.** Solution S is not more opalescent than reference suspension II (2.2.1).

**Dextrins, gum, salts, sugars.** To 2 ml of solution S, add 2 ml of alcohol R. The solution is clear. Add 1 ml of ether R. The solution remains clear for at least 10 min.

**Resins.** To 5 ml of solution S, add 5 ml of water R. The mixture remains clear for at least 15 min (2.2.1).

**Loss on drying (2.2.32).** Not more than 12.0 per cent, determined on 0.200 g by drying at 100 °C to 105 °C.

**Sulphated ash (2.4.14).** Not more than 0.1 per cent, determined on 1.0 g.

**STORAGE**
Store protected from light.

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**TARTARIC ACID**

**Acidum tartaricum**

**C₆H₈O₆**  \( M_r \) 150.1

**DEFINITION**
Tartaric acid contains not less than 99.5 per cent and not more than the equivalent of 101.0 per cent of \((2R,3R)-2,3\text{-dihydroxybutanedioic acid}\), calculated with reference to the dried substance.

**CHARACTERS**
A white or almost white, crystalline powder or colourless crystals, very soluble in water, freely soluble in alcohol.

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**TEA TREE OIL**

**Melaleucae aetheroleum**

**DEFINITION**
Essential oil obtained by steam distillation from the foliage and terminal branchlets of Melaleuca alternifolia (Maiden and Betch) Cheel, M. linearifolia Smith, M. dissitiflora F. Mueller and/or other species of Melaleuca.

**CHARACTERS**
Appearance: clear, mobile, colourless to pale yellow liquid with a characteristic odour.
IDENTIFICATION
First identification: B.
Second identification: A.

A. Thin-layer chromatography (2.2.27).

Test solution. Dissolve 0.1 ml of the substance to be examined in 5 ml of heptane R.
Reference solution. Dissolve 30 µl of cineole R, 60 µl of terpinen-4-ol R and 10 mg of aterpineol R in 10 ml of heptane R.
Plate: TLC silica gel plate R.
Application: 10 µl, as bands.
Development: over a path of 10 cm.
Drying: in air.
Detection: spray with anisaldehyde solution R. Heat at 100-105 °C for 5-10 min while observing. Examine in daylight.

Results: see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other zones are present in the chromatogram obtained with the test solution.

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results: the characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

TESTS
Relative density (2.2.5): 0.885 to 0.906.
Refractive index (2.2.6): 1.475 to 1.482.
Optical rotation (2.2.7): +5 ° to +15 °.
Chromatographic profile. Gas chromatography (2.2.28):
use the normalisation procedure.
Test solution. Dissolve 0.15 ml of the substance to be examined in 10 ml of hexane R.

Column:
- material: fused silica,
- size: t = 30 m (a film thickness of 1 µm may be used) to 60 m (a film thickness of 0.2 µm may be used),
Ω = 0.25-0.53 mm,
- stationary phase: macrogol 20 000 R.
Carrier gas: helium for chromatography R.
Flow rate: 1.3 ml/min.

Split ratio: 1.50.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1</td>
<td>50</td>
</tr>
<tr>
<td>1 - 37</td>
<td>50 → 230</td>
</tr>
<tr>
<td>37 - 45</td>
<td>230</td>
</tr>
</tbody>
</table>

Detection: flame ionisation.
Injection: 1 µl.

Elution order: order indicated in the composition of the reference solution. Record the retention times of these substances.

System suitability: reference solution:
- resolution: minimum 2.7 between the peaks due to terpinen-4-ol and aromadendrene.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution. Disregard the peak due to hexane.

Determine the percentage content of these components. The percentages are within the following ranges:
- α-pinene: 1.0 per cent to 6.0 per cent,
- sabinene: less than 3.5 per cent,
- α-terpinene: 5.0 per cent to 13.0 per cent,
- limonene: 0.5 per cent to 4.0 per cent,
- cineole: less than 15.0 per cent,
- γ-terpinene: 10.0 per cent to 28.0 per cent,
- p-cymene: 0.5 per cent to 12.0 per cent,
- terpinolene: 1.5 per cent to 5.0 per cent,
- terpinen-4-ol: minimum 30.0 per cent,
- aromadendrene: less than 7.0 per cent,
- aterpineol: 1.5 per cent to 8.0 per cent.

STORAGE
In an airtight, well-filled container, protected from light, at a temperature not exceeding 25 °C.

01/2005:0954
BETA CAROTENE
CERTIFIED REFERENCE MATERIAL

CERTIFIED PURITY: 94.9%, $U_{\text{crm}} = \pm 0.3\%$ $k = 2.09$
(Mass Balance as all-trans-Beta Carotene/as is basis)

NOMINAL PACKAGE SIZE: 1g

CATALOG #: PHR1239
LOT #: LRAA4126

CERTIFICATE VERSION: LRAA4126.1
ISSUE DATE: 27 October 2014

Note: Certificates may be updated due to Pharmacopeial Lot changes or the availability of new data.
Check our website at: www.sigma-aldrich.com for the most current version.

CRM EXPIRATION: 31 December 2018 (Proper Storage and Handling Required).

RECEIPT DATE: ____________
Note: this space is provided for convenience only and its use is not required.

STORAGE: Store in a Freezer/Protect from Light. Discard unused portions.

CHEMICAL FORMULA: $C_{40}H_{56}$
MW: 536.9

PHYSICAL DESCRIPTION: Red powder in amber vial
CAS #: 7235-40-7

HAZARDS: Read Safety Data Sheet before using. All chemical reference materials should be considered potentially hazardous and should be used only by qualified laboratory personnel.

INSTRUCTIONS FOR USE: Do not dry, use on the as is basis. The internal pressure of the container may be slightly different from the atmospheric pressure at the user’s location. Open slowly and carefully to avoid dispersion of the material. This material is intended for R&D use only. Not for drug, household or other uses.
TRACEABILITY ASSAY
Comparative assay demonstrates direct traceability to Pharmacopeial Standards

METHOD: UV (ref.: Beta Carotene, USP37)
Solvent: Cyclohexane
Cell Pathlength: 1cm
Wavelength: 457nm

ASSAY vs. USP REFERENCE STANDARD (as is basis)

<table>
<thead>
<tr>
<th>ASSAY VALUE</th>
<th>vs. USP LOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>95.1%</td>
<td>F0J240</td>
</tr>
</tbody>
</table>

Labeled Content = None, calculated= 94.2%*

*Note: The USP designates its standard content to be calculated at the time of use using the coefficient of extinction of 2505

PURITY DETERMINATION BY MASS BALANCE

CHROMATOGRAPHIC IMPURITY ANALYSIS
METHOD: HPLC (ref.: Beta Carotene, USP37)
Column: Supelcosil Suplex pkb-100, 4.6 x 250mm, 5µm
Mobile Phase: 50mg/L Butylated Hydroxytoluene, 20mL/L 2-propanol, 0.2mL/L Triethylamine, 25mL/L 0.2% Ammonium Acetate in Water, 455mL/L Acetonitrile, 460mL/L Methanol
Flow Rate: 0.6mL/min
Column Temperature: 30ºC
Injection: 20µL
Detector: 448nm

Impurities Detected:

Impurity 1: 0.2%
Impurity 2: 0.2%
Impurity 3: 0.5%
alpha Carotene: 0.7%
9-cis-beta Carotene: 0.3%
13-cis-beta Carotene: 3.2%
15-cis-beta-Carotene: 0.1%

Total Impurities: 5.1%
Representative Chromatogram from Lot: LRAA4126 Content of Beta Carotene Analysis

RESIDUAL SOLVENTS
Method: GC-MS Headspace (ref.: Residual Solvents <467>, USP34)
Column: DB-1301
Carrier gas: He
Flow: 1.2mL/min
Split Ratio: 1:5
Injection/Temperature: 1µl/250°C
Temperature Program: 40°C for 20min, 10°C/min to 240°C, hold 20min

Solvents Detected: None

LOSS ON DRYING/VOLATILES
Method: Oven at 105°C
Mean of three measurements, Loss = 0.03%

RESIDUE ANALYSIS
Method: Sulfated Ash
Sample Size: ~1g
Mean of three measurements, Residue = 0.01%

CERTIFIED PURITY BY MASS BALANCE [100% - Impurities (normalized)]

94.9% $U_{trm} = \pm 0.3\%$, $k = 2.09$
(all-trans-Beta Carotene, as is basis)
IDENTIFICATION TESTS

INFRARED SPECTROPHOTOMETRY (Comparative identification analysis demonstrates direct traceability to Pharmacopeial standards)

\[ \text{USP} \]
\[ \text{RTC} \]

\(^2\text{H NMR}\) (Data provided by an external laboratory; not in scope of accreditation)

Consistent with structure
ELEMENTAL ANALYSIS (Data provided by an external laboratory; not in scope of accreditation)
Exeter Analytical 440 Elemental Analyzer
Combustion method

<table>
<thead>
<tr>
<th>%</th>
<th>Theoretical</th>
<th>Result 1</th>
<th>Result 2</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>89.49</td>
<td>88.82</td>
<td>88.67</td>
<td>88.75</td>
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<tr>
<td>H</td>
<td>10.51</td>
<td>10.48</td>
<td>10.55</td>
<td>10.52</td>
</tr>
</tbody>
</table>

MELTING RANGE
Specification: 176-182°C (with decomposition, USP35)
Mettler Toledo FP900 Thermosystem with FP81 Measuring Cell
Mean of three measurements = 177.7-180.1°C

HOMOGENEITY ASSESSMENT
Homogeneity was assessed in accordance with ISO Guide 35. Completed units were sampled using a random stratified sampling protocol. The results of chemical analysis were then compared by Single Factor Analysis of Variance (ANOVA). The uncertainty due to homogeneity was derived from the ANOVA. Heterogeneity was not detected under the conditions of the ANOVA.

Analytical Method: UV-Vis
Sample size: ~50mg

UNCERTAINTY STATEMENT
Uncertainty values in this document are expressed as Expanded Uncertainty ($U_{crm}$) corresponding to the 95% confidence interval. $U_{crm}$ is derived from the combined standard uncertainty multiplied by the coverage factor $k$, which is obtained from a $t$-distribution and degrees of freedom. The components of combined standard uncertainty include the uncertainties due to characterization, homogeneity, long term stability, and short term stability (transport). The components due to stability are generally considered to be negligible unless otherwise indicated by stability studies.

STABILITY ASSESSMENT
Significance of the stability assessment will be demonstrated if the analytical result of the study and the range of values represented by the Expanded Uncertainty do not overlap the result of the original assay and the range of its values represented by the Expanded Uncertainty. The method employed will usually be the same method used to characterize the assay value in the initial evaluation.
Long Term Stability Evaluation - An assessment, or re-test, versus a Compendial Reference Standard may be scheduled, within the 3 year anniversary date of a release of a Secondary Standard. The re-test interval will be determined on a case-by-case basis. Short Term Stability Study - It is useful to assess stability under reasonably anticipated, short term transport conditions by simulating exposure of the product to humidity and temperature stress. This type of study is conducted under controlled conditions of elevated temperature and humidity.

APPENDIX

Original Release Date: 27 October 2014
α-TOCOPHEROL
CERTIFIED REFERENCE MATERIAL

CERTIFIED PURITY: 98.6%, $U_{crm} = \pm 0.2\%$ $k = 2$
(Mass Balance/as is basis)

NOMINAL PACKAGE SIZE: 500mg

CATALOG #: PHR1031
LOT #: P500031

CERTIFICATE VERSION: 500031.5
ISSUE DATE: 31 December 2013

Note: Certificates may be updated due to Pharmacopeial Lot changes or the availability of new data.
Check our website at: www.sigma-aldrich.com for the most current version.

CRM EXPIRATION: 12 Months from Receipt (Proper Storage and Handling Required).

RECEIPT DATE: ______________
Note: this space is provided for convenience only and its use is not required.

STORAGE: Store in a Refrigerator/Protect from Light, transfer unused portion to a tightly closed container, blanket with inert gas.

CHEMICAL FORMULA: $C_{29}H_{50}O_{2}$
MW: 430.71

PHYSICAL DESCRIPTION: Pale yellow liquid in amber ampule
CAS #: 10191-41-0

HAZARDS: Read Safety Data Sheet before using. All chemical reference materials should be considered potentially hazardous and should be used only by qualified laboratory personnel.
INSTRUCTIONS FOR USE: Do not dry, use on the as is basis. This is the dl- form of α-Tocopherol. The internal pressure of the container may be slightly different from the atmospheric pressure at the user’s location. Open slowly and carefully to avoid dispersion of the material. This material is intended for R&D use only. Not for drug, household or other uses.

TRACEABILITY ASSAY
Comparative assay demonstrates direct traceability to Pharmacopeial Standards
Specification: 96.0% to 102.0% (USP)

ASSAY vs. USP REFERENCE STANDARD (as is basis)

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<tr>
<th>ASSAY VALUE</th>
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<tbody>
<tr>
<td>98.6%</td>
<td>O0K291</td>
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</tbody>
</table>
Labeled Content = 0.985 mg/mg

METHOD: GC (ref.: all-rac-α-Tocopherol, EP6)
Column: Equity-1, 30m x 0.25mm, 0.25µm
Carrier gas: H₂
Flow: 1.4mL/min
Split Ratio: 1:20
Injection/Temperature: 1µl, 280°C
Temperature Program: 80°C for 1 min, 40°C/min to 300°C, hold 9 min
Detector/Temperature: FID, 280°C
Internal Standard: Squalane

Representative Chromatogram from Lot: P500031 USP Analysis
### ASSAY vs. EP CRS (as is basis)

<table>
<thead>
<tr>
<th>ASSAY VALUE</th>
<th>vs. EP BATCH</th>
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<tbody>
<tr>
<td>100.3%</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Labeled Content = 97.7%

**METHOD: GC (ref.: all-rac-α-Tocopherol, EP7)**
- Column: Equity-1, 30m x 0.25mm, 0.25µm
- Carrier gas: H₂
- Flow: 1.4mL/min
- Split Ratio: 20:1
- Injection/Temperature: 1µl, 290°C
- Temperature Program: 80°C for 1 min, 40°C/min to 300°C, hold 9 min
- Detector/Temperature: FID, 280°C
- Internal Standard: Squalane

Representative Chromatogram from Lot: P500031 EP Analysis

### ASSAY vs. BP CRS (as is basis)

<table>
<thead>
<tr>
<th>ASSAY VALUE</th>
<th>vs. BP BATCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>99.4%</td>
<td>3129</td>
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</tbody>
</table>

Labeled Content = 99.8%

**METHOD: GC (ref.: all-rac-α-Tocopherol, EP7)**
- Column: Equity-1, 15m x 0.53mm, 1.5µm
- Carrier gas: He
- Flow: 4.1mL/min
- Split Ratio: 1:20
- Injection/Temperature: 1µl, 280°C
- Temperature Program: 80°C for 1 min, increase 10°C/min to 280°C, hold 10 min
- Detector/Temperature: FID, 280°C
- Internal Standard: Squalane
Representative Chromatogram from Lot: P500031 BP Analysis

PURITY DETERMINATION BY MASS BALANCE
CHROMATOGRAPHIC IMPURITY ANALYSIS

METHOD: GC (ref.: all-rac-α-Tocopherol, EP6)
Column: EC-1, 30m x 0.25mm, 0.25µm
Carrier gas: He
Flow: 2mL/min
Split Ratio: 1:100
Injection/Temperature: 1µl, 290°C
Temperature Program: Hold oven at 280°C
Detector/Temperature: FID, 290°C

Impurities Detected:

Impurity A: 0.3%
Impurity B: 1.1%

Total Impurities: 1.4%

Representative Chromatogram from Lot: P500031 Impurity Analysis
RESIDUAL SOLVENTS  
Method: GC-MS Headspace (ref.: Residual Solvents <467>, USP34)  
Column: DB-1301  
Carrier gas: He  
Flow: 1.2mL/min  
Split Ratio: 1:5  
Injection/ Temperature: 1µl/250°C  
Temperature Program: 40°C for 20min, 10°C/min to 240°C, hold 20min  
Solvents Detected: None

WATER DETERMINATION  
Method: Karl Fisher titration  
Mean of three measurements, Water Content = 0.02%

RESIDUE ANALYSIS  
Method: Sulfated Ash  
Sample Size: ~1g  
Mean of three measurements, Residue = 0.04%

CERTIFIED PURITY BY MASS BALANCE  
[100% - Impurities (normalized)]  
98.6%  $U_{CRM} = \pm 0.2\%$, $k = 2$  
(as is basis)

IDENTIFICATION TESTS

INFRARED SPECTROPHOTOMETRY  
(Comparative identification analysis demonstrates direct traceability to Pharmacopeial standards)
\[ ^2H \text{ NMR} \] (Data provided by an external laboratory; not in scope of accreditation)

\[ ^{13} \text{C} \text{ NMR} \] in CDCl3

Consistent with structure

\[ \text{ELEMENTAL ANALYSIS} \] (Data provided by an external laboratory; not in scope of accreditation)

Exeter Analytical 440 Elemental Analyzer

Combustion method

<table>
<thead>
<tr>
<th>%</th>
<th>Theoretical</th>
<th>Result 1</th>
<th>Result 2</th>
<th>Mean</th>
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<tr>
<td>C</td>
<td>80.87</td>
<td>81.03</td>
<td>81.08</td>
<td>81.06</td>
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<tr>
<td>H</td>
<td>11.70</td>
<td>11.68</td>
<td>11.62</td>
<td>11.65</td>
</tr>
</tbody>
</table>

\[ \text{OPTICAL ROTATION} \]

Specification: -0.01° to +0.01° (EP)

Perkin Elmer Polarimeter 343

Wavelength: 589nm

Concentration: 10g/100mL in EtOH

Cell Path: 100mm

Mean of three Measurements = -0.004°
HOMOGENEITY ASSESSMENT
Homogeneity was assessed in accordance with ISO Guide 35. Completed units were sampled using a random stratified sampling protocol. The results of chemical analysis were then compared by Single Factor Analysis of Variance (ANOVA). The uncertainty due to homogeneity was derived from the ANOVA. Heterogeneity was not detected under the conditions of the ANOVA.

Analytical Method: GC  Sample size: ~100 mg

UNCERTAINTY STATEMENT
Uncertainty values in this document are expressed as Expanded Uncertainty (U_{crm}) corresponding to the 95% confidence interval. U_{crm} is derived from the combined standard uncertainty multiplied by the coverage factor k, which is obtained from a t-distribution and degrees of freedom. The components of combined standard uncertainty include the uncertainties due to characterization, homogeneity, long term stability, and short term stability (transport). The components due to stability are generally considered to be negligible unless otherwise indicated by stability studies.

STABILITY ASSESSMENT
Significance of the stability assessment will be demonstrated if the analytical result of the study and the range of values represented by the Expanded Uncertainty do not overlap the result of the original assay and the range of its values represented by the Expanded Uncertainty. The method employed will usually be the same method used to characterize the assay value in the initial evaluation.

Long Term Stability Evaluation - An assessment, or re-test, versus a Compendial Reference Standard may be scheduled, within the 3 year anniversary date of a release of a Secondary Standard. The re-test interval will be determined on a case-by-case basis.

Short Term Stability Study - It is useful to assess stability under reasonably anticipated, short term transport conditions by simulating exposure of the product to humidity and temperature stress. This type of study is conducted under controlled conditions of elevated temperature and humidity.

Operations Manager     QA Supervisor
APPENDIX

Original Release Date: 17 September 2009
Stability Test Date: 22 November 2011
Requalification Test Date: 22 November 2011
Requalification Test Date: 17 December 2012
Requalification Test Date: 18 December 2013
Certificate of Analysis

Product Number: 147524
Batch Number: SHBD4790V
Brand: ALDRICH
CAS Number: 80-56-8
MDL Number: MFCD00001339
Formula: C10H16
Formula Weight: 136.23 g/mol
Quality Release Date: 06 FEB 2014

<table>
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<tr>
<th>Test</th>
<th>Specification</th>
<th>Result</th>
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<td>Appearance (Color)</td>
<td>Colorless</td>
<td>Colorless</td>
</tr>
<tr>
<td>Appearance (Form)</td>
<td>Liquid</td>
<td>Liquid</td>
</tr>
<tr>
<td>Infrared Spectrum</td>
<td>Conforms to Structure</td>
<td>Conforms</td>
</tr>
<tr>
<td>Purity (GC)</td>
<td>≥ 97.5 %</td>
<td>98.9 %</td>
</tr>
<tr>
<td>Optical Rotation (Neat)</td>
<td>-0.20 - 0.20 °</td>
<td>0.00 °</td>
</tr>
</tbody>
</table>

Jamie Gleason, Manager
Quality Control
Sheboygan Falls, WI  US

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.
Certificate of Analysis

Product Name: α-Terpine – 85%

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<th>Product Number:</th>
<th>223182</th>
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</thead>
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<td>Batch Number:</td>
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<tr>
<td>Brand:</td>
<td>ALDRICH</td>
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<tr>
<td>CAS Number:</td>
<td>99-86-5</td>
</tr>
<tr>
<td>MDL Number:</td>
<td>MFCD00001534</td>
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<tr>
<td>Formula:</td>
<td>C10H16</td>
</tr>
<tr>
<td>Formula Weight:</td>
<td>136.23 g/mol</td>
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<tr>
<td>Quality Release Date:</td>
<td>22 NOV 2013</td>
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</table>

<table>
<thead>
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<th>Test</th>
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<th>Result</th>
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</thead>
<tbody>
<tr>
<td>Appearance (Color)</td>
<td>Colorless</td>
<td>Colorless</td>
</tr>
<tr>
<td>Appearance (Form)</td>
<td>Liquid</td>
<td>Liquid</td>
</tr>
<tr>
<td>Infrared Spectrum</td>
<td>Conforms to Structure</td>
<td>Conforms</td>
</tr>
<tr>
<td>Purity (GC)</td>
<td>≥ 84.5 %</td>
<td>91.3 %</td>
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<tr>
<td>Solubility (Turbidity)</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>100mg/ml, Ethanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solubility (Color)</td>
<td>Colorless to Pale Yellow</td>
<td>Colorless</td>
</tr>
</tbody>
</table>

Jamie Gleason, Manager
Quality Control
Milwaukee, Wisconsin  US

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# Certificate of Analysis

**Product Name:**  (+)-α-TERPINEOL analytical standard

**Product Number:**  83073  
**Batch Number:**  BCBN0603V  
**Brand:**  Fluka  
**CAS Number:**  7785-53-7  
**Formula:**  C_{10}H_{18}O  
**Formula Weight:**  154.25  
**Storage Temperature:**  2-8 C  
**Quality Release Date:**  16 JUL 2014

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<td>APPEARANCE (COLOR)</td>
<td>COLORLESS TO VERY FAINT YELLOW AND COLORLESS TO VERY FAINT BROWN-YELLOW</td>
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<tr>
<td>APPEARANCE (FORM)</td>
<td>CLEAR LIQUID OR SOLID</td>
<td>LIQUID</td>
</tr>
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<td>PURITY (GC AREA %)</td>
<td>≥ 97.0 %</td>
<td>97.1 %</td>
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<td>SPECIFIC ROTATION (20/D)</td>
<td>84.0 ± 10.0 DEGREES</td>
<td>89.0 DEGREES</td>
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<td>CONCENTRATION</td>
<td>NEAT</td>
<td>NEAT</td>
</tr>
<tr>
<td>PROTON NMR SPECTRUM</td>
<td>CONFORMS TO STRUCTURE</td>
<td>CONFORMS</td>
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</tbody>
</table>

Dr. Claudia Geitner  
Manager Quality Control  
Buchs, Switzerland  

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Certificate of Analysis

<table>
<thead>
<tr>
<th>TEST</th>
<th>SPECIFICATION</th>
<th>RESULT</th>
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<tbody>
<tr>
<td>APPEARANCE (COLOR)</td>
<td>COLORLESS TO VERY FAINTLY YELLOW</td>
<td>VERY FAINTLY YELLOW (Y6)</td>
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<td>APPEARANCE (FORM)</td>
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<td>CLEAR LIQUID</td>
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<td>PURITY (GC AREA %)</td>
<td>≥ 97.0 %</td>
<td>98.8 %</td>
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<td>SPECIFIC ROTATION (20/D)</td>
<td>12.0 1.0 DEGREES</td>
<td>12.8 DEGREES</td>
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<td>1.496 - 1.498</td>
<td>1.498</td>
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</tbody>
</table>

Dr. Claudia Geitner
Manager Quality Control
Buchs, Switzerland

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Certificate of Analysis

Product Name: 1,4-CINEOLE
technical, mixture of isomers, >= 85 % GC

Product Number: 27395
Batch Number: BCBM1534V
Brand: Aldrich
CAS Number: 470-67-7
Formula: C₁₀H₁₈O
Formula Weight: 154.25
Quality Release Date: 13 NOV 2013

TEST | SPECIFICATION | RESULT
--- | --- | ---
APPEARANCE (COLOR) | COLORLESS TO VERY FAINT YELLOW | COLORLESS
APPEARANCE (FORM) | LIQUID | LIQUID
PURITY (GC AREA %) | ≥ 70.0 % | 92.5 %
GC MINOR COMPONENT 1 (AREA %) | ≤ 20 % | 1.2 %
MINOR COMPONENT 1 | 1.8-CINEOLE | 1.8-CINEOLE
REFRACTIVE INDEX N20/D | 1.447 - 1.455 | 1.447
PROTON NMR SPECTRUM | CONFORMS TO STRUCTURE | CONFORMS

Dr. Claudia Geitner
Manager Quality Control
Buchs, Switzerland

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**Certificate of Analysis**

**Product Name:** γ-Terpinene – 97%

**Product Number:** 223190  
**Batch Number:** MKBN9731V  
**Brand:** ALDRICH  
**CAS Number:** 99-85-4  
**MDL Number:** MFCD00001537  
**Formula:** C10H16  
**Formula Weight:** 136.23 g/mol  
**Quality Release Date:** 11 MAR 2013

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<tr>
<td>Appearance (Form)</td>
<td>Liquid</td>
<td>Liquid</td>
</tr>
<tr>
<td>Infrared spectrum</td>
<td>Conforms to Structure</td>
<td>Conforms</td>
</tr>
<tr>
<td>Purity (GC)</td>
<td>≥ 96.5 %</td>
<td>99.3 %</td>
</tr>
</tbody>
</table>

Jamie Gleason, Manager  
Quality Control  
Milwaukee, Wisconsin US

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Certificate of Analysis

Product Name: (S)-(−)-Limonene - 96%

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<td>Brand:</td>
<td>ALDRICH</td>
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<td>CAS Number:</td>
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<td>MDL Number:</td>
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<td>Formula:</td>
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<tr>
<td>Formula Weight:</td>
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<td>28 APR 2011</td>
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<table>
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<tr>
<td>Appearance (Color)</td>
<td>Colorless</td>
<td>Colorless</td>
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<tr>
<td>Appearance (Form)</td>
<td>Liquid</td>
<td>Liquid</td>
</tr>
<tr>
<td>Infrared spectrum</td>
<td>Conforms to Structure</td>
<td>Conforms</td>
</tr>
<tr>
<td>Optical Rotation C= Neat</td>
<td>-90 - -61deg</td>
<td>-82deg</td>
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<tr>
<td>Purity (GC)</td>
<td>&gt; 95.5%</td>
<td>97.9%</td>
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</tbody>
</table>

Jamie Gleason, Manager
Quality Control
Milwaukee, Wisconsin US

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Certificate of Analysis

Product Name: p-Cymene - 99%

Product Number: C121452
Batch Number: MKBP8457V
Brand: ALDRICH
CAS Number: 99-87-6
MDL Number: MFCD00008893
Formula: C10H14
Formula Weight: 134.22 g/mol
Quality Release Date: 30 JUL 2013

<table>
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<td>Appearance (Color)</td>
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<tr>
<td>Appearance (Form)</td>
<td>Liquid</td>
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<tr>
<td>Infrared spectrum</td>
<td>Conforms to Structure</td>
<td>Conforms</td>
</tr>
<tr>
<td>Purity (GC)</td>
<td>≥ 98.5 %</td>
<td>99.6 %</td>
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</tbody>
</table>

Jamie Gleason, Manager
Quality Control
Milwaukee, Wisconsin US

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Certificate of Analysis

Product Name: (−)-TERPINEN-4-OL  
>= 95.0 % GC sum of enantiomers

Product Number: 11584
Batch Number: BCBM5331V
Brand: Aldrich
CAS Number: C_10 H_18 O
Formula: 154.25
Formula Weight: 28 JAN 2014

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<td>APPEARANCE (COLOR)</td>
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<td>COLORLESS</td>
</tr>
<tr>
<td>APPEARANCE (FORM)</td>
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<td>LIQUID</td>
</tr>
<tr>
<td>PURITY (GC AREA %)</td>
<td>≥ 95.0 %</td>
<td>99.5 %</td>
</tr>
<tr>
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<td>CONCENTRATION</td>
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<td>PROTON NMR SPECTRUM</td>
<td>CONFORMS TO STRUCTURE</td>
<td>CONFORMS</td>
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</table>

Dr. Claudia Geitner
Manager Quality Control
Buchs, Switzerland

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Certificate of Analysis

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<tr>
<td>APPEARANCE (FORM)</td>
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<td>LIQUID</td>
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<tr>
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