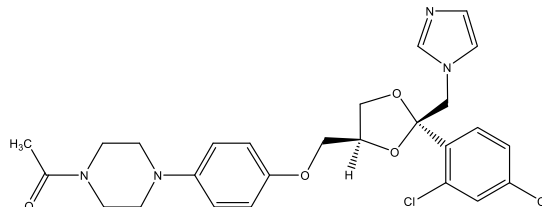


Certificate of Analysis – Certified Reference Material

KETOCONAZOLE

Product no.: PHR1385-1G
Lot no.: LRAC3465
Description of CRM: White Powder
Expiry date: 31 August 2023
Storage: Room Temperature
Certificate version: LRAC3465.2 (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.)
Chemical formula: C₂₆H₂₈Cl₂N₄O₄
Molecular mass: 531.4
CAS No.: 65277-42-1



Analyte	Certified Purity ± associated uncertainty U , $U = k \cdot u$ ($k =$) (Mass Balance/basis)
KETOCONAZOLE	99.3 % Ucrm = ± 0.2 %, $k = 2.14$ (Mass Balance/as is basis)

Metrological traceability: Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. Additional traceability to Primary Standards is established through comparative assay determinations. See "Details on metrological traceability" on page 2.
Measurement method: Where applicable, the certified value is based on a purity determination by mass balance. See "Certification process details" on page 3.
Intended use: Intended for R&D and Analytical Use only. Not for drug, household or other uses.
Minimum sample size: 40 mg
Instructions for handling and correct 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. Attachment of a 20 mm aluminum crimp seal recommended for unused portions.
Health and safety information: All chemical reference materials should be considered potentially hazardous and should be used only by qualified laboratory personnel. Please refer to the Safety Data Sheet for detailed information about the nature of any hazard and appropriate precautions to be taken.
Accreditation: Sigma-Aldrich RTC is accredited by the US accreditation authority ANAB as a registered reference material producer AR-1470 in accordance with ISO 17034.
Certificate issue date: 31 July 2020



[Andy Ommen; Quality Control]

[Mark Pooler; Quality Assurance]



Packaging: 1 g in amber vial

Details on metrological traceability: This standard has been gravimetrically prepared using balances that have been fully qualified and calibrated to ISO 17025 requirements. All calibrations utilize NIST traceable weights which are calibrated externally by a qualified ISO 17025 accredited calibration laboratory to NIST standards. Qualification of each balance includes the assignment of a minimum weighing by a qualified and ISO 17025 accredited calibration vendor taking into consideration the balance and installed environmental conditions to ensure compliance with USP tolerances of NMT 0.10% relative error. Fill volume to predetermined specifications is gravimetrically verified throughout the dispensing process using qualified and calibrated balances. Further traceability to a corresponding Primary Standard may be achieved through a direct comparison assay. Where a Primary Standard is available, the assay value will be included in the specified section of the COA.

Associated uncertainty: 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.

Traceability Assay:

Comparative assay demonstrates direct traceability to Pharmacopeial Standards

ASSAY vs. USP REFERENCE STANDARD (as is basis)

<u>ASSAY VALUE</u>	<u>vs. USP LOT</u>
98.8 %	H1F086
	Labeled Content = 0.998 mg/mg

ASSAY vs. BP CRS (as is basis)

<u>ASSAY VALUE</u>	<u>vs. BP BATCH</u>
99.0 %	3788
	Labeled Content = 100.0 %

Method: HPLC (ref.: Ketoconazole Oral Solution, Current Compendial Monographs)

Column: Ascentis Express C18, 100 mm x 4.6 mm x 5 μ m

Mobile Phase: 0.01M Tetrabutylammonium Hydrogen Sulfate in Water, Acetonitrile (75:25)

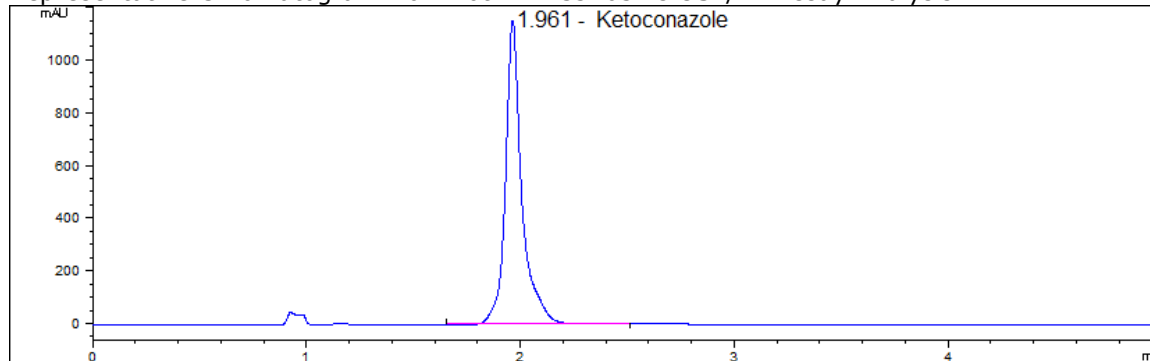
Flow Rate: 1 mL/min

Column Temperature: 30 $^{\circ}$ C

Injection: 5 μ L

Detector: 223 nm

Representative Chromatogram from Lot: LRAC3465 vs USP/BP Assay Analysis



ASSAY vs. EP CRS (as is basis)

ASSAY VALUE
98.4 %

vs. EP BATCH
3.0

Labeled Content = None

Assigned Content = 99.348 % *

*The assigned content of the EP CRS was determined by assay against the USP Reference Standard

Method: HPLC (ref.: Ketoconazole Oral Solution, Current Compendial Monographs)

Column: Ascentis Express C18, 100 mm x 4.6 mm x 5 µm

Mobile Phase: 0.01M Tetrabutylammonium Hydrogen Sulfate in Water, Acetonitrile (75:25)

Flow Rate: 1 mL/min

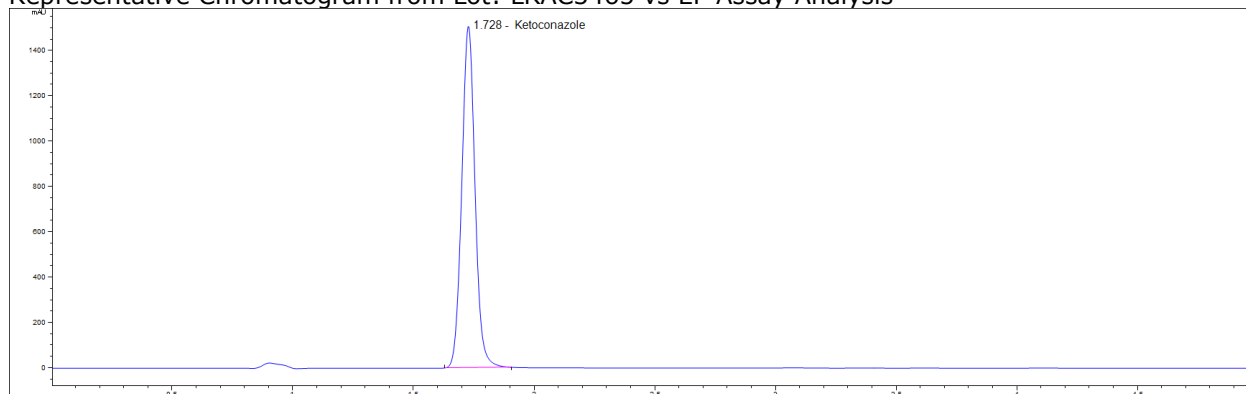
Column Temperature: 30 °C

Injection: 5 µL

Detector: VWD

Wavelength: 223 nm

Representative Chromatogram from Lot: LRAC3465 vs EP Assay Analysis



Certification process details:

The certified purity is determined by mass balance and calculated as

$$\% \text{ Purity} = \left(\frac{(100 - TCI)}{100} * \frac{(100 - LOD)}{100} * \frac{(100 - H_2O)}{100} * \frac{(100 - ROI)}{100} * \frac{(100 - RS)}{100} \right) * 100\%$$

- TCI = Total Chromatographic Impurities
- LOD = Loss on Drying
- H₂O = Water content determined by Karl Fischer analysis
- ROI = Residue on Ignition
- RS = Residual Solvents

Methods for impurity determination may be added or deleted as required. The following techniques are applied:

CHROMATOGRAPHIC IMPURITY ANALYSIS

METHOD: HPLC (ref.: Ketoconazole, Current Compendial Monographs)

Column: Ascentis Express C18, 100 mm x 4.6 mm x 5 µm

Mobile Phase A: 3.4 g/L Tetrabutylammonium hydrogen sulfate in Water, Acetonitrile (95:5)

Mobile Phase B: 3.4 g/L Tetrabutylammonium hydrogen sulfate in Water, Acetonitrile (50:50)

Gradient:

Time (min)	% A	% B
0-10	100-0	0-100
10-15	0	100
15-17	0-100	100-0

Flow Rate: 2 mL/min

Column Temperature: 30 °C

Injection: 5 µL

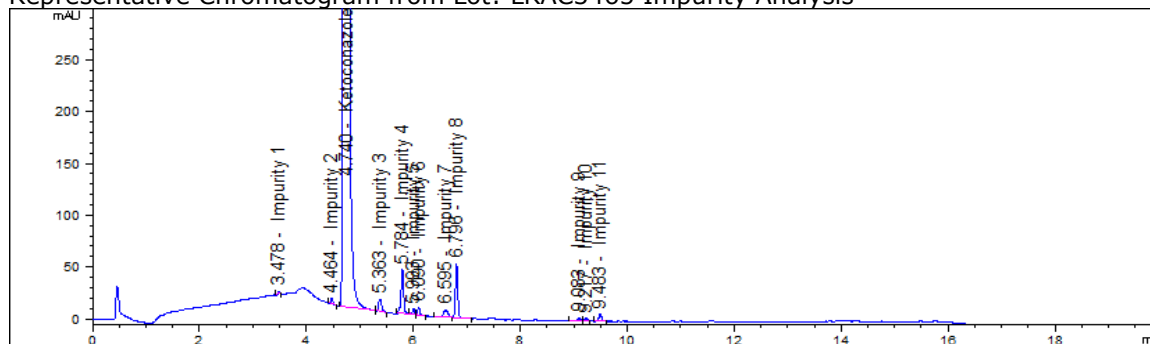
Detector: 220 nm

Impurities Detected:

Impurity 1:	0.009 %
Impurity 2:	0.02 %
Impurity 3:	0.06 %
Impurity 4:	0.2 %
Impurity 5:	0.02 %
Impurity 6:	0.03 %
Impurity 7:	0.05 %
Impurity 8:	0.2 %
Impurity 9:	0.01 %
Impurity 10:	0.01 %
Impurity 11:	0.03 %

Total Impurities: **0.6 %**

Representative Chromatogram from Lot: LRAC3465 Impurity Analysis



RESIDUAL SOLVENTS

Method: GC-MS Headspace (ref.: Adapted from Residual Solvents USP <467>)

Column: SPB-624

Carrier gas: He

Flow: 10.2 mL/min

Split Ratio: 1:5

Injection/Temperature: 1 µL/250 °C

Temperature Program: 40 °C for 5 min, 8 °C/min to 200°C, hold 5 min

Solvents Detected: **None**

LOSS ON DRYING/VOLATILES

Method: In a vacuum at 80 °C (ref.: Current Compendial Monographs)

Mean of three measurements, Loss = **0.1 %**

RESIDUE ANALYSIS

Method: Sulfated Ash (ref.: Current Compendial Monographs)

Sample Size: ~ 1 g

Mean of three measurements, Residue = **0.02 %**

CERTIFIED PURITY BY MASS BALANCE

99.3 % $U_{\text{crm}} = \pm 0.2 \%$, $k = 2.14$
(as is basis)

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: HPLC

Sample size: 40 mg

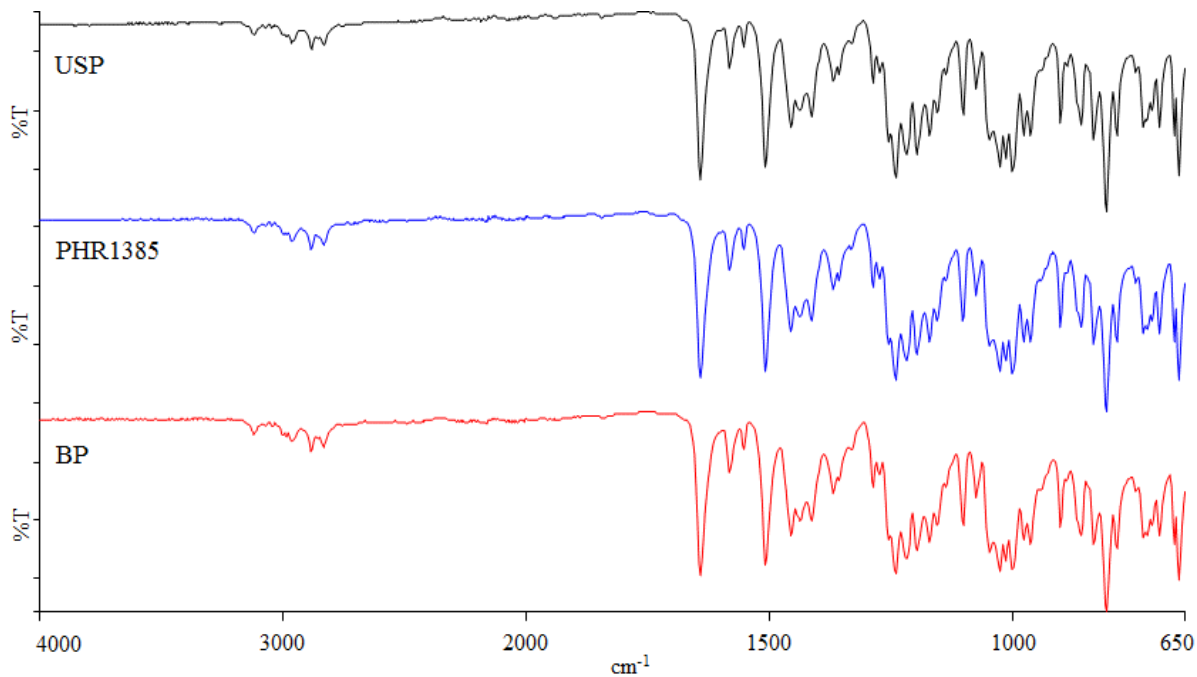
Stability assessment:

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.

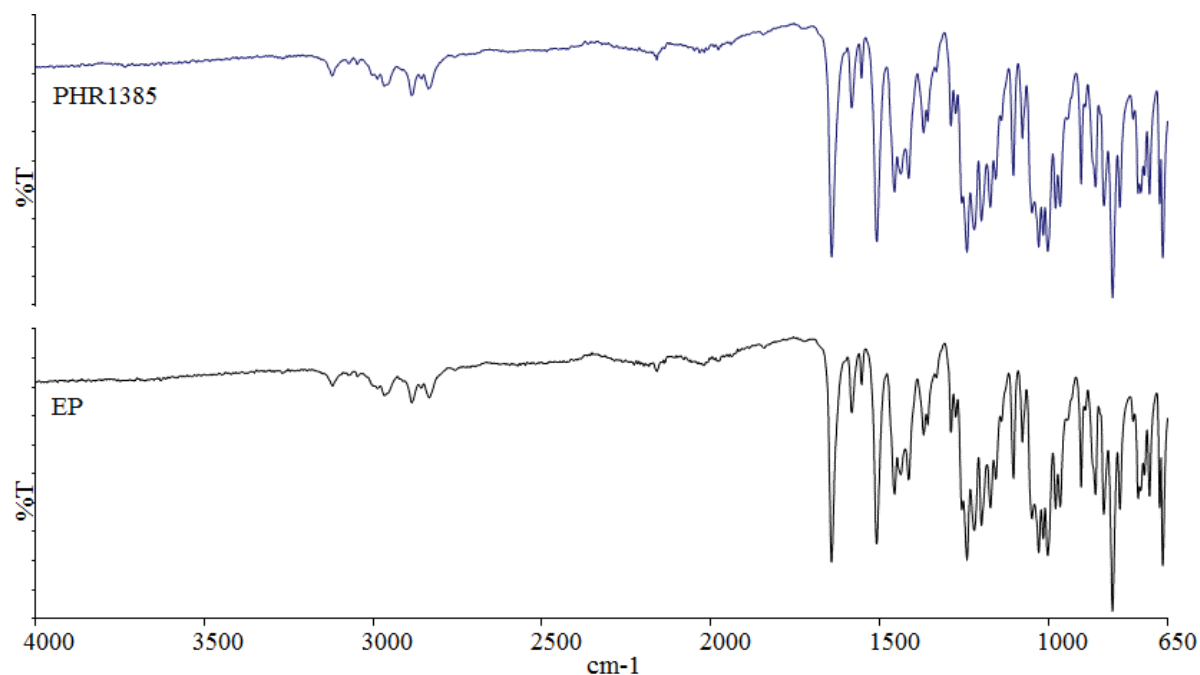
Long Term Stability Evaluation - An assessment, or re-test, versus a Compendial Reference Standard may be scheduled, within the 3 year anniversary date of a release of a Secondary Standard. The re-test interval will be determined on a case-by-case basis. Short Term Stability Study - It is useful to assess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

Identification Test:

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



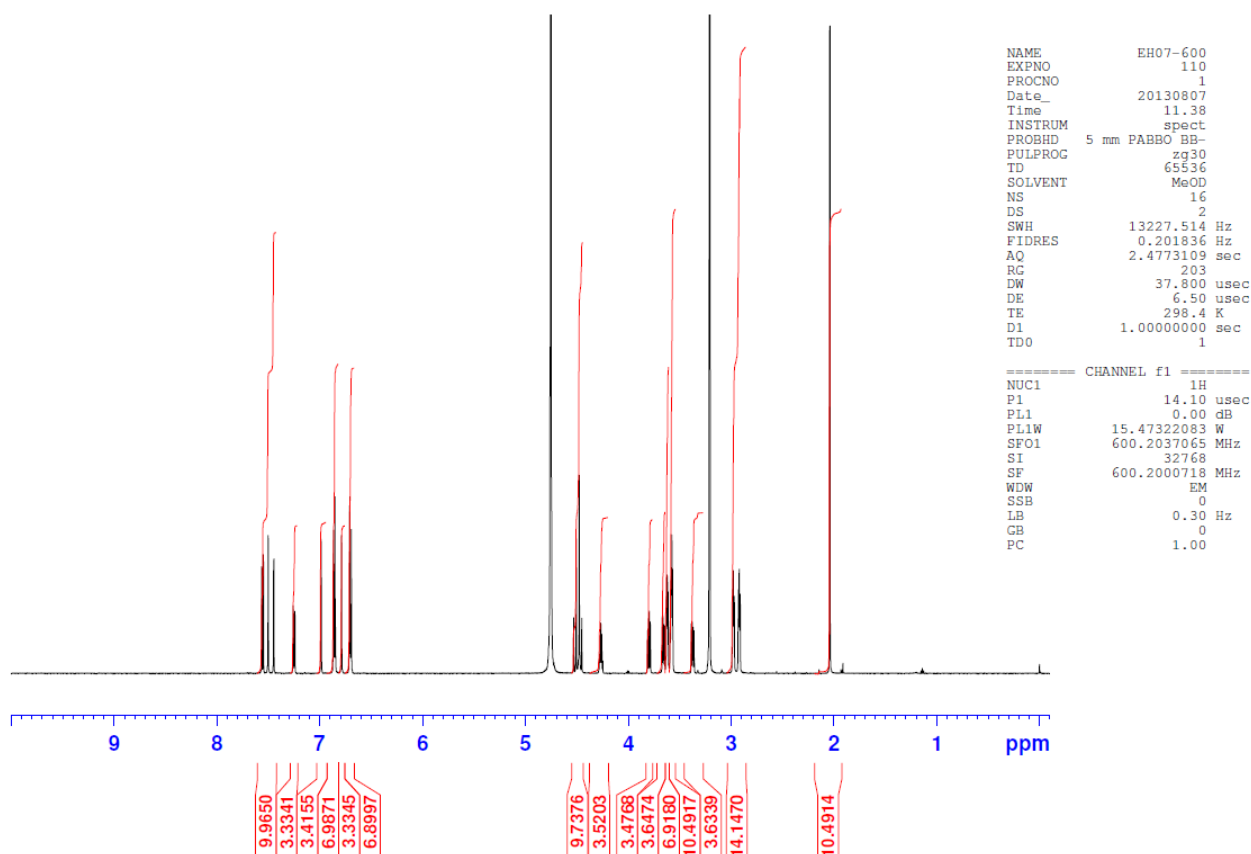
Ketoconazole USP Lot H1F086 vs PHR1385 LRAC3465 vs BP Batch 3788



Ketoconazole PHR1385 LRAC3465 vs EP Batch 3.0

Indicative Values:

¹H NMR (Data provided by an external laboratory; not in scope of accreditation)
LRAC3465 Ketoconazole



Consistent with structure

ELEMENTAL ANALYSIS (Data provided by an external laboratory; not in scope of accreditation)

Exeter Analytical 440 Elemental Analyzer

Combustion method

%	Theoretical	Result 1	Result 2	Mean
C	58.76	58.63	58.64	58.64
H	5.31	5.12	5.05	5.09
N	10.54	10.44	10.34	10.39

MELTING RANGE

Specification: 148-152 °C (USP)

Mettler Toledo FP900 Thermosystem with FP81 Measuring Cell

Mean of three measurements = **148.3-148.9 °C**

OPTICAL ROTATION

Specification: -1° to +1°

Perkin Elmer Polarimeter 343

Wavelength: 589 nm

Concentration: ~ 4 g/100 mL

Cell Path: 100 mm

Mean of three Measurements = **0.0028°**

Certificate of analysis revision history:

Certificate version	Date	Reason for version
LRAC3465.1	07 August 2019	Original Release
LRAC3465.2	31 July 2020	Stability & Requalification Test

Disclaimer:

The purchaser is required to determine the suitability of this product for any particular application. Sigma-Aldrich RTC makes no warranty of any kind, express or implied, other than its products meet all quality control standards set by Sigma-Aldrich RTC. We do not guarantee that the product can be used for any particular application.

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