

CERTIFICATE OF ANALYSIS

ERM[®] - AC316a

Solvent Yellow 124 - *N*-ethyl-*N*-[2-(1-isobutoxyethoxy)ethyl]-4-(phenylazo)aniline

Parameter	Certified value ¹ (mass %)	Uncertainty ² (mass %)
Purity	95.0	1.2

1) The certified value was obtained by combining the data from HPLC-UV and GC-FID (both using peak area normalisation) and is traceable to the SI.

2) The quoted uncertainty is the expanded uncertainty calculated using a coverage factor (*k*) of 2.15, which gives a level of confidence of approximately 95 %.

This certificate is valid for 12 months from the date of shipment provided the sample is stored under the recommended conditions.

The minimum amount of sample to be used is 4 mg.

NOTE

European Reference Material ERM[®] - AC316a was produced and certified under the responsibility of LGC according to the principles laid down in the Technical Guidelines of the European Reference Materials[®] co-operation agreement between BAM-LGC-IRMM. Information on these guidelines is available on the Internet (<http://www.erm-crm.org>).

Accepted as an ERM[®], Teddington, April 2008.

Signed: _____

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All following pages are an integral part of the certificate.

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DESCRIPTION OF THE SAMPLE

A batch of Solvent Yellow 124 (SY124) was obtained from a commercial source and purified by column chromatography. The purified material was homogenised in propan-2-ol by stirring for 2 hours at room temperature. The solvent was subsequently removed by evaporation using a rotary evaporator and its purity was assessed by LGC.

The material was considered to be homogeneous on the basis of measurements made by gas chromatography with flame ionisation detection (GC-FID), on 10 randomly selected 4 mg portions, which showed no significant variation in purity value.

The identity of the material was confirmed by 500 MHz ¹H-NMR spectroscopy.

The material was dispensed as 200 mg units in screw cap 4 mL amber glass vials.

INTENDED USE

The material is intended for the use as an analytical standard for the determination of SY124 in fuel.

ANALYTICAL METHOD USED FOR CERTIFICATION

The purity assessment of the material was carried out by LGC using two chromatographic methods ie high performance liquid chromatography with UV detection (HPLC-UV) and GC-FID.

High Performance Liquid Chromatography with UV Detection (HPLC-UV)

Reverse phase HPLC-UV was carried out using the experimental conditions described in Tables 1 & 2.

Table 1: HPLC experimental conditions

LC system	Waters Separations Module 2695
Analytical column	Phenomenex, Luna, Phenyl-Hexyl 150 x 2 mm; 5 µm
Wavelength	202.8 nm
Injection volume	10 µL
Flow	0.3 mL/min

A mobile phase gradient was used (Table 2) to analyse solvent-free SY124.

Table 2: Gradient used for reverse phase HPLC analysis of SY124

Time (min)	Flow (mL/min)	Water (%)	Methanol (%)	Acetonitrile (%)
0	0.3	30	20	50
35	0.3	30	20	50
45	0.3	30	0	70
60	0.3	0	0	100
65	0.3	0	0	100
70	0.3	30	20	50
75	0.3	30	20	50

The purity assessment of ERM-AC316a using HPLC-UV was carried out at 202.8 nm (2nd λ_{max}) because most of the impurities are not detectable at the λ_{max} of SY124 (423 nm).

Gas Chromatography with Flame Ionisation Detection (GC-FID)

GC-FID was carried out using the experimental conditions described in Table 3.

Table 3: GC conditions used for the analysis of solvent-free SY124

GC system	Thermo Trace 2000 GC-FID
Processing software	Chromcard software version 2.01
Analytical column	Fused silica/100 % dimethyl polysiloxane, 60 m x 0.25 mm i.d., film thickness 0.25 µm
Injection system	Cold-on-column
Injection volume	2 µL
Carrier gas	Helium (1 mL/min)
Oven temperature program	100 °C-120 °C at 45 °C/min 120 °C-230 °C at 5 °C/min 230 °C-285 °C at 1.5 °C/min – hold for 5 min 285 °C-310 °C at 5 °C/min – hold for 15 min
Total run time	84.11 minutes

Moisture

Moisture was determined by ambient and oven coulometric Karl Fischer titration and found to be 0.071 % m/m. This value was subtracted from the mean organic purity determined by GC-FID and HPLC-UV. An apura® 1 % oven standard (nominal water content: 1.00 ± 0.03 mass %, Merck, Darmstadt, Germany) and 0.1 % Hydranal standard (0.1 ± 0.003 mass %, Hydranal, Riedel-de Haën, Sigma Aldrich, Seelze, Germany) were used as QC standards to check instrument performance.

Inorganic Content

The percentage of inorganic material was assessed by ashing. About 2.5 g of sample (n = 3) was weighed into a crucible and heated from ambient temperature to 650 °C over 3 hours. The inorganic content (mass %) was calculated as the weight of residue after completion of the ashing and the initial sample weight and found to be negligible.

Table 4 shows very good agreement between SY124 purity determined by HPLC-UV and GC-FID. The mean value for moisture was subtracted from the mean values for HPLC-UV and GC-FID.

Table 4

Purity (mass %)					
	mean	n	s.d.	Corrected mean	s.e.m
GC-FID	95.04	20	0.14	94.97 ¹	0.032
HPLC-UV	95.10	20	0.20	95.03 ¹	0.045
Mean of means	95.07	2	0.05	95.00 ¹	0.035
Total standard uncertainty for organic purity					0.064
Uncertainty contribution for low purity (< 98 mass %)					0.5
Uncertainty contribution from moisture determination					0.005
Uncertainty contribution from ashing					0.007
Uncertainty contribution from possible inhomogeneity					0.12
Uncertainty contribution from possible instability					0.12
Total standard uncertainty					0.53
Effective degrees of freedom (d.o.f.)					14.68
t for 14.94 d.o.f and 95 % confidence					2.15
Expanded uncertainty (k = 2.15) at 95 % confidence					1.2
1) Corrected mean is the mean minus the mean moisture content (0.071 mass %)					
n = number of observations					
s.d = standard deviation					
s.e.m = standard error (uncertainty) of corrected mean					

The certified purity value is the mean of the corrected individual means of the individual methods as shown in Table 4. The expanded uncertainty of the purity value was calculated by multiplying the total standard uncertainty by the t-value for the number of effective degrees of freedom for the certified value. The expanded uncertainty gives a level of confidence of approximately 95 %.

PARTICIPANTS

Not applicable.

SAFETY INFORMATION

Refer to material safety data sheet.

INSTRUCTIONS FOR USE

Analytical standards using ERM-AC316a should be prepared with a minimum of 10 mg and dissolved in a suitable solvent for analysis. For this work, HPLC analysis was carried out using SY124 dissolved in methanol and in GC-FID analysis carried out using SY124 dissolved in hexane.

STORAGE

The material should be stored at $5\text{ }^{\circ}\text{C} \pm 4\text{ }^{\circ}\text{C}$ in the original closed vial until it is required for use. Once opened, the closed vial should be stored at $5\text{ }^{\circ}\text{C} \pm 4\text{ }^{\circ}\text{C}$ in a secondary container with a desiccant to prevent the ingress of moisture.

SPECIMEN

Unit Number:

Shipment Date:

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