C 042001

Title

Endosulfan and Metabolites
PARTITION COEFFICIENT
1-OCTANOL/WATER
(HPLC-Method)

Endosulfan hydroxy carboxylic acid, sodium salt (AE F114151)
Endosulfan diol (AE F051329)
Endosulfan hydroxy ether (AE F051326)
Endosulfan lactone (AE F051328)
Endosulfan sulfate (AE F051327)
Endosulfan ether (AE F051330)
beta-Endosulfan (AE F052619)
alpha-Endosulfan (AE F052618)

Authors

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Test Guideline

92/69/EEC A.8

OECD 117

Completed on

June 21, 2004

Testing facility

Bayer CropScience GmbH

Product Technology-Analytics Frankfurt

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Federal Republic of Germany

Study Identification

PA04/036

CONFIDENTIALITY STATEMENT

This report is confidential. No part of the report or any information contained therein may be disclosed to any third party without the prior written authorisation of Bayer CropScience.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with the *Principles of Good Laboratory Practice* as adopted by the Council of OECD on 26th November, 1997 [(C97) 186/Final] as implemented at the national level.

Study Director: **B. Mühlberger**

signature

21.06.04
date (ddMONyyyy)

Head of
Testing Facility: **Dr. M. Feyerabend**

signature

4.6.04
date (ddMONyyyy)

Dr. Müller

Quality Assurance (GLP)

Quality Assurance Statement

Title: **Endosulfan and Metabolites**
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beta-Endosulfan (AE F052619)
alpha-Endosulfan (AE F052618)

Study: PA04/036

This study was periodically inspected and properly signed records of these inspections were submitted to testing facility management and the study director as shown below.
This report has been audited by the GLP Quality Assurance. The reported results accurately reflect the original data of the study.

Phase of Study	Inspection	Report
Study plan	13.04.2004	13.04.2004
Study conduct	14.04.2004	14.04.2004
Study plan amendment	16.04.2004	16.04.2004
	09.06.2004	09.06.2004
Draft report	19.06.2004 - 21.06.2004	21.06.2004
Study plan amendment	21.06.2004	21.06.2004
Final report	21.06.2004	21.06.2004

21.06.2004

A. Winterstein
GLP Quality Assurance

APPROVALS PAGE

Study Director: **B. Mühlberger** — signature — 21.06.04 — date (ddMONyyyy)

Experimental work coordinated by: **G. Lemke** — signature — 21/6/04 — date (ddMONyyyy)

Head of Testing Facility: **Dr. M. Feyerabend** — signature — Dr. Müller — 21.6.04 — date (ddMONyyyy)

Audited by Quality Assurance: 21.06.2004

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SUMMARY

Study title: Endosulfan and Metabolites
PARTITION COEFFICIENT 1-OCTANOL/WATER (HPLC-Method)

Authors: B. Mühlberger, G. Lemke,

Test Guideline: 92/69/EEC, appendix, A.8, OECD 117

The partition coefficient (1-octanol/water) of the test items were determined according to OECD guideline 117 (dated, March 30, 1989) and EEC-guideline 92/69/EEC (dated, July 31, 1992) A.8, HPLC-method. Therefore, 9 neutral calibration substances were injected into a HPLC-system under the same analytical conditions (column temperature 40°C) as the test items. A calibration curve was created by using the measured retention times (log k'-values) and the known log P_{ow}-values of the calibration substances for linear regression.
The measured log k'-value of the test items were within the calibrated log k'-range.

From the resulted calibration curve and its equation the log P_{ow} of the substances had been interpolated.

The partition coefficient (1-octanol / water) of the test items were:

Table 1:

Test item	Log P_{ow}	P_{ow}
Endosulfan hydroxy carboxylic acid, sodium salt, AE F114151 [1)]	**2.05**	**112**
Endosulfan diol, AE F051329	**2.92**	**832**
Endosulfan hydroxy ether, AE F051326	**3.22**	**1660**
Endosulfan lactone, AE F051328	**3.40**	**2512**
Endosulfan sulfate, AE F051327	**3.77**	**5888**
Endosulfan ether, AE F051330	**4.15**	**14125**
beta-Endosulfan, AE F052619	**4.34**	**21878**
alpha-Endosulfan, AE F052618	**4.63**	**42658**

[1)] Test performed under acid conditions

STUDY IDENTIFICATION

Sponsor:	Bayer CropScience GmbH Product Technology-Analytics Frankfurt D-65926 Frankfurt am Main, FRG
Testing Facility:	Bayer CropScience GmbH Product Technology-Analytics Frankfurt D-65926 Frankfurt am Main, FRG
Study Director:	B. Mühlberger Product Technology-Analytics Frankfurt
Experimental work coordinated by:	G. Lemke Product Technology-Analytics Frankfurt
Quality Assurance:	Dr. J. Celorio GLP Quality Assurance Bayer CropScience GmbH D-65926 Frankfurt am Main, FRG
Study No.:	PA 04/036
Study Timetable	
Study initiation date:	April 13, 2004
Experimental start date:	April 14, 2004
Experimental termination date:	April 20, 2004
Study completion date:	June 21, 2004
Archive:	Bayer CropScience GmbH Product Technology-Analytics Frankfurt D-65926 Frankfurt am Main, FRG

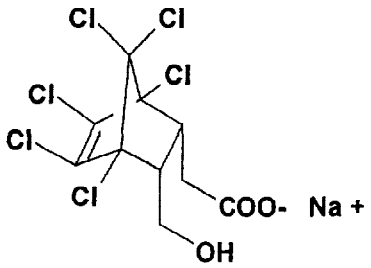
1. Objective

The objective of this study was the determination of the partition coefficient 1-octanol/water of the test items according to guideline 92/69/EEC (dated, July 31, 1992), appendix, A.8; OECD 117 (dated, March 30, 1989).

2. Test Item

2.1. AE F114151

Test item:	AE F114151; Substance, pure
Chemical name:	sodium 1,4,5,6,7,7-hexachloro-3-hydroxymethyl-bicyclo[2.2.1]hept-5-en-2-carboxylate
Trivial name:	Endosulfan hydroxy carboxylic acid, sodium salt
Sample code:	AE F114151 00 1B97 0001
Batch:	RDB3969
Structure:	

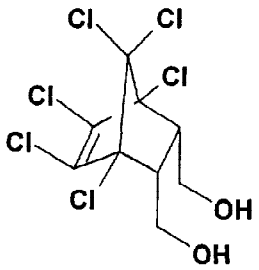


Empirical formula:	$C_9H_5Cl_6NaO_3$
Molecular weight:	396,83 g/mol
Purity:	96,6 % (w/w)
Certificate:	AZ 11488, dated April 8, 2004
Expiry date:	March 25, 2007

2.2. AE F051329

Test item:	AE F051329; Substance, pure
Chemical name:	1,4,5,6,7,7-hexachloro-bicyclo-(2,2,1)-hept-5-ene-2,3-dimethanol
Trivial name:	Endosulfan diol
Sample code:	AE F051329 00 1B99 0005
Batch:	C02352073

Structure:

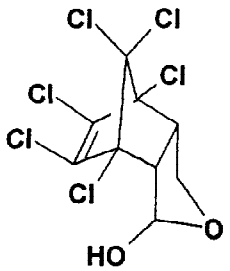


Empirical formula:	$C_9H_8Cl_6O_2$
Molecular weight:	360,87 g/mol
Purity:	99,6 % (w/w)
Certificate:	AZ 08445, dated May 04, 2000
Expiry date:	April 27, 2004

2.3. AE F051326

Test item:	AE F051326; Substance, pure
Chemical name:	4,5,6,7,8,8-hexachloro-1,3,3a,4,7,7a-hexahydro-4,7-methano-isobenzo-furan-1-ol
Trival name:	Endosulfan hydroxy ether
Sample code:	AE F051326 00 1B96 0002
Batch:	REW 1008-7

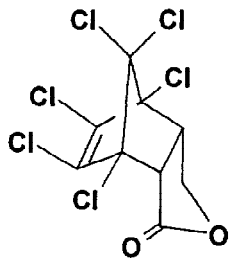
Structure:



Empirical formula:	$C_9H_6Cl_6O_2$
Molecular weight:	358,85 g/mol
Purity:	95,0 % (w/w)
Certificate:	AZ 09689, dated December 20, 2001
Expiry date:	December 18, 2005

2.4. AE F051328

Test item:	AE F051328; Substance, pure
Chemical name:	4,5,6,7,8,8-hexachloro-1,3,3a,4,7,7a-hexahydro-4,7-methano-isobenzo-furan-1-one
Trivial name:	Endosulfan lactone
Sample code:	AE F051328 00 1B95 0001
Batch:	DJA 119
Structure:	

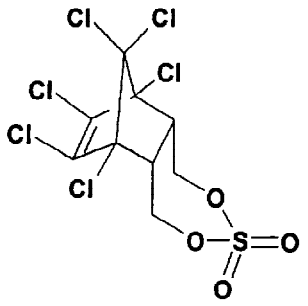


Empirical formula:	$C_9H_4Cl_6O_2$
Molecular weight:	356,83 g/mol
Purity:	95,1 % (w/w)
Certificate:	AZ 010186, dated October 18, 2002
Expiry date:	October 9, 2006

2.5. AE F051327

Test item:	AE F051327; Substance, pure
Chemical name:	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3,3-dioxide
Trivial name:	Endosulfan sulfate
Sample code:	AE F051327 00 1B99 0005
Batch:	GHTD340/12

Structure:



Empirical formula: $C_9H_6Cl_6O_4S$

Molecular weight: 422,91 g/mol

Purity: 99,7 % (w/w)

Certificate: AZ 09755, dated February 1, 2002

Expiry date: January 25, 2006

2.6. AE F051330

Test item: AE F051330; Substance, pure

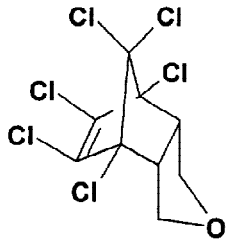
Chemical name: 4,5,6,7,8,8-hexachloro-1,3,3a,4,7,7a-hexahydro-4,7-methano-isobenzo-furan

Trivial name: Endosulfan ether

Sample code: AE F051330 00 1B99 0004

Batch: 23510

Structure:



Empirical formula: $C_9H_6Cl_6O$

Molecular weight: 342,85 g/mol

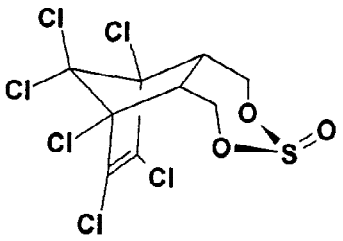
Purity: 99,2 % (w/w)

Certificate: AZ 09170, dated May 10, 2001

Expiry date: May 7, 2006

2.7. AE F052619

Test item:	AE F052619; Substance, pure
Chemical name:	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo-dioxathiepin-3-oxide-beta-isomer
Trivial name:	beta-Endosulfan
Sample code:	AE F052619 00 1B99 0007
Batch:	RU141/1 1.Fällung
Structure:	

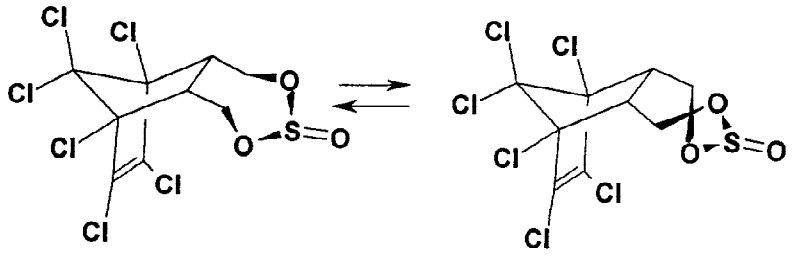


Empirical formula:	$C_9H_6Cl_6O_3S$
Molecular weight:	406,91 g/mol
Purity:	99,6 % (w/w)
Certificate:	AZ 10821, dated July 2, 2003
Expiry date:	July 1, 2007

2.8. AE F052618

Test item:	AE F052618; Substance, pure
Chemical name:	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo-dioxathiepin-3-oxide-alpha-isomer
Trivial name:	alpha-Endosulfan
Sample code:	AE F052618 00 1B99 0010
Batch:	R142/7

Structure:



first twist chair form **second twist chair form**

The two isomeric forms are not distinguishable at room temperature. They were identified by X ray spectrometry.

Empirical formula:	$C_9H_6Cl_6O_3S$
Molecular weight:	406,91 g/mol
Purity:	99,1 % (w/w)
Certificate:	AZ 09487, dated October 2, 2001
Expiry date:	September 25, 2007

3. Performance of the HPLC Method

3.1. Principle

The HPLC method is based on the correlation between the retention of a substance on a reversed phase column and the 1-octanol/water partition coefficient P_{OW}.

$$\log P_{OW} = a + b \log k' \qquad (1)$$

$$k' = \frac{t_R - t_0}{t_0} \quad \text{(capacity factor)} \qquad (2)$$

with t_R retention time of substances
t_0 dead time

The dead time is determined by using a homologous series of n-alkyl methyl ketones. For calibration according to equation (1) retention times of selected reference compounds with known 1-octanol/water partition coefficients are measured. In a further step the log P_{OW} of the test item should be derived from its retention time.

3.2. Reagents

- Acetonitrile, Chromasolv (Riedel de Haen)
- Millipore water
- n-Alkyl methyl ketones (Fluka; Table 2, 5)
- Calibration substances (Aldrich, Fluka; Table 3, 6)
- Phosphoric acid, 85 % (Riedel de Haen)

3.3. Instruments

- Chromatographic apparatus refer chapter 3.4
- General laboratory glassware: flasks, etc.
- Analytical balance

3.4. Chromatographic Conditions

Except for the detection wavelength used by the UV detector the chromatographic conditions were identical for the determination of t_0, the establishment of the calibration graph, and the measurement of the test item.

HPLC equipment:	Agilent HPLC system with autosampler G1313A, pump G1312A, column oven G1316A, UV/VIS detector G1314A; Software Chromeleon, Fa. Dionex Version 6.40 SP1	
Column:	Material: stainless steel (Bischoff) dimensions: 250 x 3.0 mm stationary phase: Hypersil ODS, 5 µm column temperature: 40°C	
Mobile Phase:	mobile phase for neutral conditions: isocratic mixture of acetonitrile / water (70:30) (v/v); (pH 7.3) mobile phase for acid conditions: isocratic mixture of acetonitrile/ 0.01M phosphoric acid (70:30) (v/v); (pH 2.5), for test item AE F114151	
Flow rate:	1.0 ml/min	
Injected volume:	20 µl	
Detection wavelength:	275 nm (determination of t_0), 254 nm (calibration graph) 220 nm (analysis of the test items)	
Injection amounts:	n-Alkyl methyl ketones: Calibration substances: Test items:	160.0 - 188.4 µg 0.22 - 105.48 µg 2.09 - 3.32 µg

The alkyl ketones, calibration substances and the test items, respectively, were injected in duplicate as solutions in acetonitrile.

3.5. Dead Time t_0

The dead time was determined by simultaneous injection of the n-alkyl methyl ketones shown in Table 2 and 5.

Using the slope and the intercept, t_0 can be calculated from a linear regression.

$$t_R(n_C + 1) = a + b\, t_R(n_C) \qquad (3) \qquad (n_C : \text{no. of the homologue})$$

$$t_0 = a/(1 - b) \qquad (4)$$

The data and the evaluation are given in Table 2, 5, 8 and 11 (chapter 4) and Figure 1 and 3 (chapter 6), respectively.

3.6. Calibration and Measurement

For calibration the retention times of a series of "neutral" substances with known 1-octanol/water partition coefficients were measured and a calibration graph according to equation (1) was established (Figure 2 and 4, chapter 6).
The 1-octanol/water partition coefficient of the test item was derived from its retention time and the calibration graph.

Individual results and the evaluation are given in Tables 4, 7, 10 and 13 (chapter 4).

4. Experimental Data

4.1. Experimental Data

Table 2: Determination of the dead time t_0 (neutral conditions)

RUN 1

Ketone	n_C	t_R (min) 1. Injection	t_R (min) 2. Injection	t_R (min) Mean
2-Butanone	4	1.60	1.60	1.600
2-Pentanone	5	1.70	1.70	1.700
2-Hexanone	6	1.83	1.83	1.830
2-Heptanone	7	2.07	2.03	2.050
2-Octanone	8	2.37	2.37	2.370
2-Nonanone	9	2.80	2.80	2.800
2-Decanone	10	3.43	3.43	3.430
2-Undecanone	11	4.37	4.37	4.370
2-Dodecanone	12	5.77	5.77	5.770
2-Tridecanone	13	7.80	7.80	7.800

Regression: $t_R(n_C + 1) = a + b\ t_R(n_C)$

Parameters:

$a = -0.6501$

$b = 1.4649$

$r^2 = 0.9999$

Dead time:

$t_0 = a / (1 - b)$

$t_0 = 1.398$ min

Table 3: Calibration data (* from OECD guideline 117, adopted March 1989) (neutral conditions)

RUN 1

Calibration substance	1. Inj. t_R (min)	2. Inj. t_R (min)	Mean t_R (min)	k'	log k'	log P_{OW} *
2-Butanone	1.60	1.60	1.600	0.1445	-0.8402	0.30
Acetanilide	1.53	1.53	1.530	0.0944	-1.0249	1.00
Cinnamic alcohol	1.67	1.67	1.670	0.1946	-0.7109	1.90
2,6-Dichlorobenzonitrile	2.13	2.13	2.130	0.5236	-0.2810	2.60
Allylphenylether	2.33	2.33	2.330	0.6667	-0.1761	2.90
Benzophenone	2.43	2.33	2.380	0.7024	-0.1534	3.20
Isopropylbenzene	3.20	3.20	3.200	1.2890	0.1102	3.70
Diphenylether	3.00	2.97	2.985	1.1352	0.0551	4.20
Fluoranthene	4.50	4.50	4.500	2.2189	0.3461	4.70

Regression: $\log P_{OW} = a + b \log k'$

Parameters:

a	=	3.6110
b	=	2.9901
r^2	=	0.9215

Table 4: Log P_{OW} of the test items (neutral conditions)

RUN 1

Test item	1. Inj. t_R (min)	2. Inj. t_R (min)	Mean t_R (min)	k'	log k'	log P_{OW}
AE F114151	1.13	1.10	1.115	-0.2024	-	< 0 [1)]
AE F051329	2.23	2.20	2.215	0.5844	-0.2333	2.91
AE F051326	2.43	2.43	2.430	0.7382	-0.1318	3.22
AE F051328	2.57	2.60	2.585	0.8491	-0.0711	3.40
AE F051327	2.97	2.97	2.970	1.1245	0.0509	3.76
AE F051330	3.50	3.50	3.500	1.5036	0.1771	4.14
AE F052619	3.83	3.83	3.830	1.7396	0.2405	4.33
AE F052618	4.43	4.43	4.430	2.1688	0.3362	4.62

[1)] estimated

Table 5: Determination of the dead time t_0 (neutral conditions)

RUN 2

Ketone	n_C	t_R (min) 1. Injection	t_R (min) 2. Injection	t_R (min) Mean
2-Butanone	4	1.60	1.60	1.600
2-Pentanone	5	1.70	1.70	1.700
2-Hexanone	6	1.83	1.83	1.830
2-Heptanone	7	2.03	2.03	2.030
2-Octanone	8	2.37	2.37	2.370
2-Nonanone	9	2.80	2.80	2.800
2-Decanone	10	3.43	3.43	3.430
2-Undecanone	11	4.37	4.37	4.370
2-Dodecanone	12	5.77	5.77	5.770
2-Tridecanone	13	7.80	7.80	7.800

Regression: $t_R(n_C + 1) = a + b\, t_R(n_C)$

Parameters:

$a = -0.6483$

$b = 1.4647$

$r^2 = 0.9999$

Dead time:

$t_0 = a / (1 - b)$

$t_0 = 1.395$ min

Table 6: Calibration data (* from OECD guideline 117, adopted March 1989) (neutral conditions)

RUN 2

Calibration substance	1. Inj. t_R (min)	2. Inj. t_R (min)	Mean t_R (min)	k'	log k'	log P_{OW} *
2-Butanone	1.60	1.60	1.600	0.1470	-0.8328	0.30
Acetanilide	1.53	1.53	1.530	0.0968	-1.0142	1.00
Cinnamic alcohol	1.67	1.67	1.670	0.1971	-0.7052	1.90
2,6-Dichlorobenzonitrile	2.13	2.13	2.130	0.5269	-0.2783	2.60
Allylphenylether	2.33	2.33	2.330	0.6703	-0.1738	2.90
Benzophenone	2.33	2.33	2.330	0.6703	-0.1738	3.20
Isopropylbenzene	3.20	3.20	3.200	1.2939	0.1119	3.70
Diphenylether	2.97	2.97	2.970	1.1290	0.0527	4.20
Fluoranthene	4.50	4.50	4.500	2.2258	0.3475	4.70

Regression: $\log P_{OW} = a + b \log k'$

Parameters:

a	=	3.6156
b	=	3.0160
r^2	=	0.9205

Table 7: Log P_{OW} of the test items (neutral conditions)

RUN 2

Test item	1. Inj. t_R (min)	2. Inj. t_R (min)	Mean t_R (min)	k'	log k'	log P_{OW}
AE F114151	1.07	1.07	1.070	-0.2330	-	< 0 [1)]
AE F051329	2.23	2.20	2.215	0.5878	-0.2308	2.92
AE F051326	2.43	2.43	2.430	0.7419	-0.1296	3.22
AE F051328	2.57	2.57	2.570	0.8423	-0.0745	3.39
AE F051327	2.97	2.97	2.970	1.1290	0.0527	3.77
AE F051330	3.50	3.50	3.500	1.5090	0.1787	4.15
AE F052619	3.83	3.83	3.830	1.7455	0.2419	4.35
AE F052618	4.47	4.43	4.450	2.1900	0.3404	4.64

[1)] estimated

Table 8: Determination of the dead time t_0 for AE F114151 (acid conditions)

RUN 1

Ketone	n_C	t_R (min) 1. Injection	t_R (min) 2. Injection	t_R (min) Mean
2-Butanone	4	1.60	1.60	1.600
2-Pentanone	5	1.70	1.70	1.700
2-Hexanone	6	1.83	1.83	1.830
2-Heptanone	7	2.03	2.03	2.030
2-Octanone	8	2.37	2.37	2.370
2-Nonanone	9	2.80	2.80	2.800
2-Decanone	10	3.43	3.43	3.430
2-Undecanone	11	4.37	4.37	4.370
2-Dodecanone	12	5.77	5.73	5.750
2-Tridecanone	13	7.77	7.77	7.770

Regression: $t_R(n_C + 1) = a + b\ t_R(n_C)$

Parameters:

$a = -0.6449$

$b = 1.4627$

$r^2 = 0.9999$

Dead time:

$t_0 = a / (1 - b)$

$t_0 = 1.394$ min

Table 9: Calibration data (* from OECD guideline 117, adopted March 1989) for AE F114151 (acid conditions)

RUN 1

Calibration substance	1. Inj. t_R (min)	2. Inj. t_R (min)	Mean t_R (min)	k'	log k'	log P_{OW} *
2-Butanone	1.60	1.60	1.600	0.1478	-0.8304	0.30
Acetanilide	1.53	1.57	1.550	0.1119	-0.9511	1.00
Cinnamic alcohol	1.67	1.67	1.670	0.1980	-0.7034	1.90
2,6-Dichlorobenzonitrile	2.13	2.13	2.130	0.5280	-0.2774	2.60
Allylphenylether	2.33	2.33	2.330	0.6714	-0.1730	2.90
Benzophenone	2.33	2.33	2.330	0.6714	-0.1730	3.20
Isopropylbenzene	3.20	3.20	3.200	1.2956	0.1125	3.70
Diphenylether	2.97	3.00	2.985	1.1413	0.0574	4.20
Fluoranthene	4.50	4.50	4.500	2.2281	0.3479	4.70

Regression: $\log P_{OW} = a + b \log k'$

Parameters:

a	=	3.6177
b	=	3.1112
r^2	=	0.9298

Table 10: Log P_{OW} of the test item AE F114151 (acid conditions)

RUN 1

Test item	1. Inj. t_R (min)	2. Inj. t_R (min)	Mean t_R (min)	k'	log k'	log P_{OW}
AE F114151	1.83	1.83	1.830	0.3128	-0.5048	2.05

Table 11: Determination of the dead time t_0 for AE F114151 (acid conditions)

RUN 2

Ketone	n_C	t_R (min) 1. Injection	t_R (min) 2. Injection	t_R (min) Mean
2-Butanone	4	1.60	1.60	1.600
2-Pentanone	5	1.70	1.70	1.700
2-Hexanone	6	1.83	1.83	1.830
2-Heptanone	7	2.07	2.07	2.070
2-Octanone	8	2.37	2.37	2.370
2-Nonanone	9	2.80	2.80	2.800
2-Decanone	10	3.43	3.43	3.430
2-Undecanone	11	4.37	4.37	4.370
2-Dodecanone	12	5.77	5.77	5.770
2-Tridecanone	13	7.77	7.80	7.785

Regression: $t_R(n_C + 1) = a + b\ t_R(n_C)$

Parameters:

$a = -0.6456$

$b = 1.4624$

$r^2 = 0.9999$

Dead time:

$t_0 = a / (1 - b)$

$t_0 = 1.396$ min

Table 12: Calibration data (* from OECD guideline 117, adopted March 1989) for AE F114151 (acid conditions)

RUN 2

Calibration substance	1. Inj. t_R (min)	2. Inj. t_R (min)	Mean t_R (min)	k'	log k'	log P_{OW} *
2-Butanone	1.60	1.60	1.600	0.1461	-0.8353	0.30
Acetanilide	1.53	1.57	1.550	0.1103	-0.9574	1.00
Cinnamic alcohol	1.67	1.67	1.670	0.1963	-0.7071	1.90
2,6-Dichlorobenzonitrile	2.17	2.13	2.150	0.5401	-0.2675	2.60
Allylphenylether	2.33	2.33	2.330	0.6691	-0.1745	2.90
Benzophenone	2.33	2.33	2.330	0.6691	-0.1745	3.20
Isopropylbenzene	3.20	3.20	3.200	1.2923	0.1114	3.70
Diphenylether	2.97	2.97	2.970	1.1275	0.0521	4.20
Fluoranthene	4.50	4.50	4.500	2.2235	0.3470	4.70

Regression: $\log P_{OW} = a + b \log k'$

Parameters:

a	=	3.6194
b	=	3.0986
r^2	=	0.9283

Table 13: Log P_{OW} of the test item AE F114151 (acid conditions)

RUN 2

Test item	**1. Inj. t_R (min)**	**2. Inj. t_R (min)**	**Mean t_R (min)**	**k'**	**log k'**	**log P_{OW}**
AE F114151	1.83	1.83	1.830	0.3109	-0.5074	2.05

Table 14:

Test item	RUN 1 Log P_{ow}	RUN 2 Log P_{ow}
Endosulfan hydroxy carboxylic acid, sodium salt, AE F114151 [1)]	2.05	2.05
Endosulfan diol, AE F051329	2.91	2.92
Endosulfan hydroxy ether, AE F051326	3.22	3.22
Endosulfan lactone, AE F051328	3.40	3.39
Endosulfan sulfate, AE F051327	3.76	3.77
Endosulfan ether, AE F051330	4.14	4.15
beta-Endosulfan, AE F052619	4.33	4.35
alpha-Endosulfan, AE F052618	4.62	4.64

[1)] Test performed under acid conditions

5. Results and Conclusion

The partition coefficient (1-octanol/water) of the test items were determined according to OECD guideline 117 (dated, March 30, 1989) and EEC-guideline 92/69/EEC (dated, July 31, 1992) A.8, HPLC-method. Therefore, 9 neutral calibration substances were injected into a HPLC-system under the same analytical conditions (column temperature 40°C) as the test item. A calibration curve was created by using the measured retention times (log k'-values) and the known log P_{ow}-values of the calibration substances for linear regression.
and EEC-guideline 92/69/EWG A.8,
The measured log k'-value of the test items were within the calibrated log k'-range. From the resulted calibration curve and its equation the log P_{ow} of the test items had been interpolated.

The partition coefficient (1-octanol / water) of the test item is:

Table 15:

Test item	Log P_{ow}	P_{ow}
Endosulfan hydroxy carboxylic acid, sodium salt, AE F114151 [1)]	**2.05**	**112**
Endosulfan diol, AE F051329	**2.92**	**832**
Endosulfan hydroxy ether, AE F051326	**3.22**	**1660**
Endosulfan lactone, AE F051328	**3.40**	**2512**
Endosulfan sulfate, AE F051327	**3.77**	**5888**
Endosulfan ether, AE F051330	**4.15**	**14125**
beta-Endosulfan, AE F052619 [2)]	**4.34**	**21878**
alpha-Endosulfan, AE F052618 [2)]	**4.63**	**42658**

[1)] Test performed under acid conditions

[2)] The determination of the partition coefficient octanol/water by the "Flask Shaking Method" resulted in a Log Pow of 4.79 for beta-Endosulfan (AE F052619) and 4.74 for alpha-Endosulfan (AE F052618), respectively.
Report: Hoe 052618 and Hoe 052619 alpha- and beta-Endosulfan, Partition Coefficient Octanol/Water; Authors: Dr. R. Sarafin, Dr. J. Aßhauer; Study-No.: (B) 124/87 Hoechst Aktiengesellschaft, Analytisches Laboratorium; AGREDOC-No.: A36576.

The results of the HPLC-method are within the variation limit given by the OECD *Guideline for* Testing of Chemicals No. 117, page 2:
"The inter-laboratory comparison test has shown that with the HPLC method log Pow values can be obtained to within ±0.5 units of the Shake-Flask values."

6. Figures

Figure 1: Determination of t_0 - RUN 1 (neutral conditions)

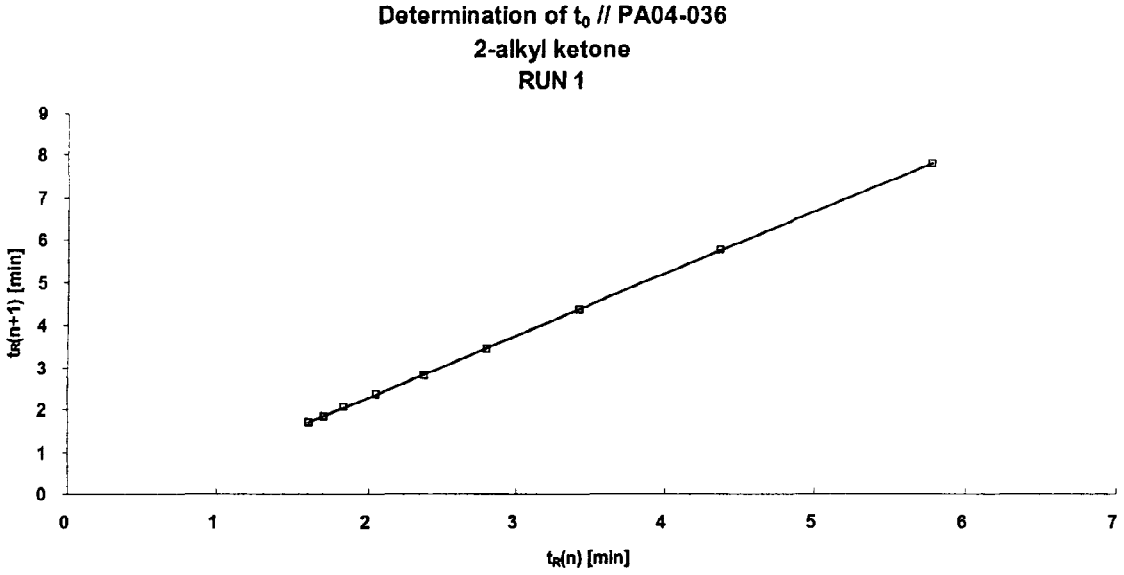


Figure 2: Calibration graph log k' versus log P_{OW} of the calibration substances - RUN 1 (neutral conditions)

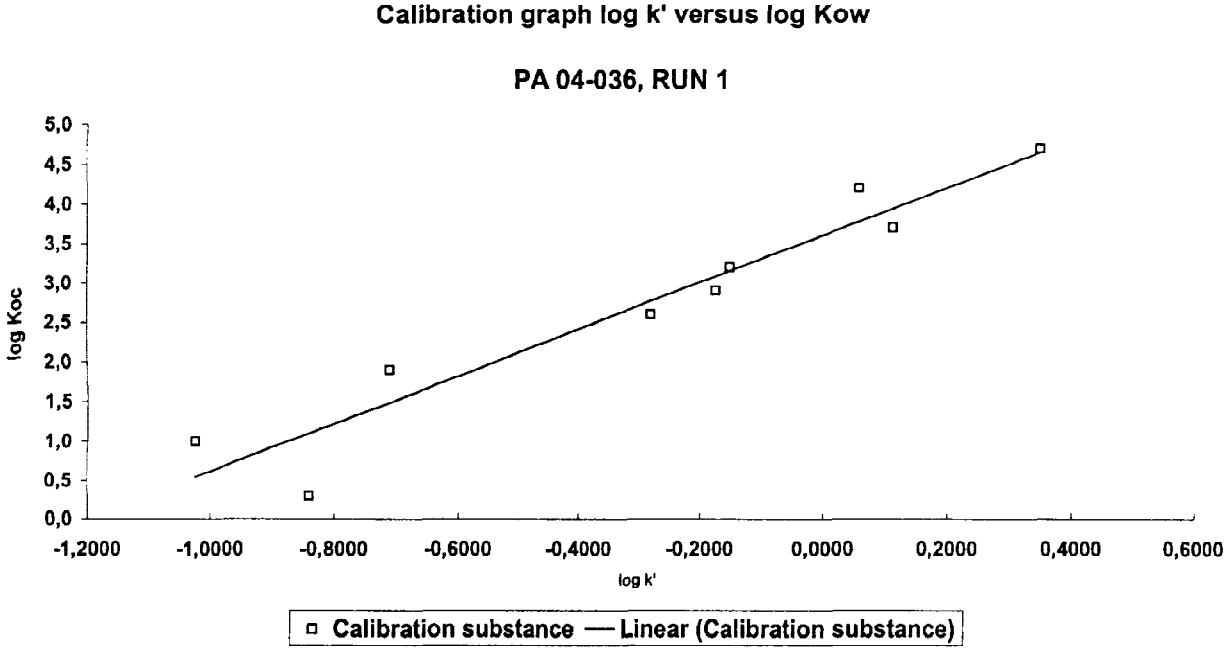


Figure 3: Determination of t_0 - RUN 1 (acid conditions)

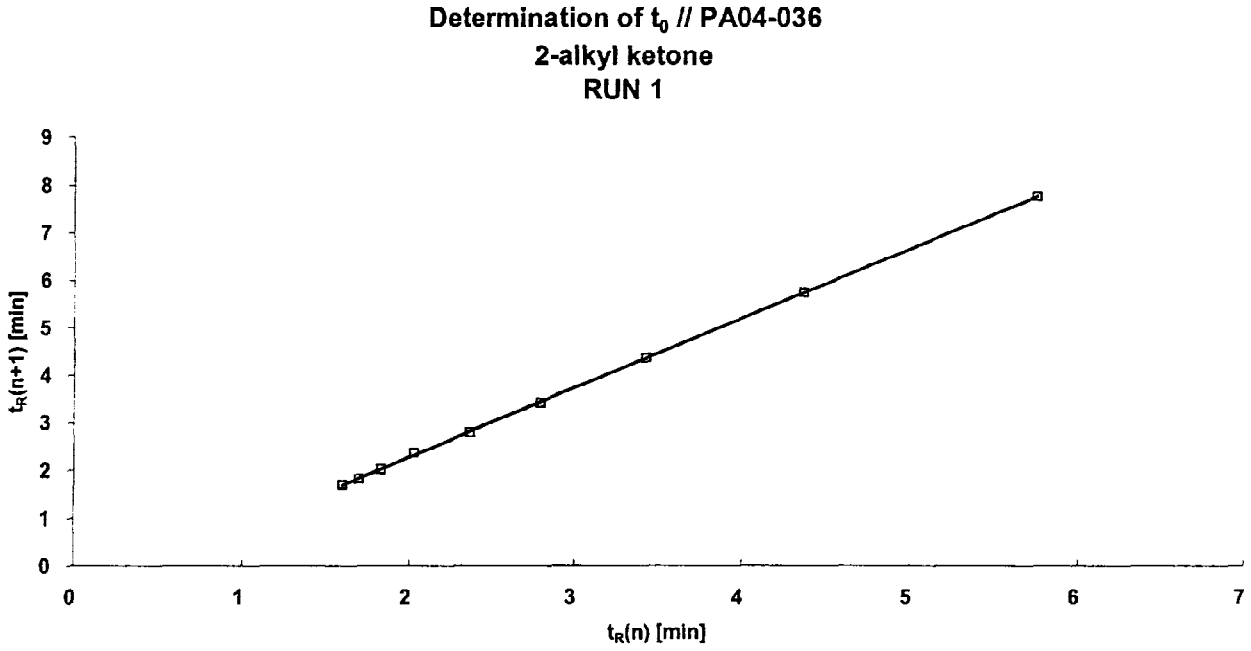


Figure 4: Calibration graph log k' versus log P_{OW} of the calibration substances, for AE F114151 - RUN 1 (acid conditions)

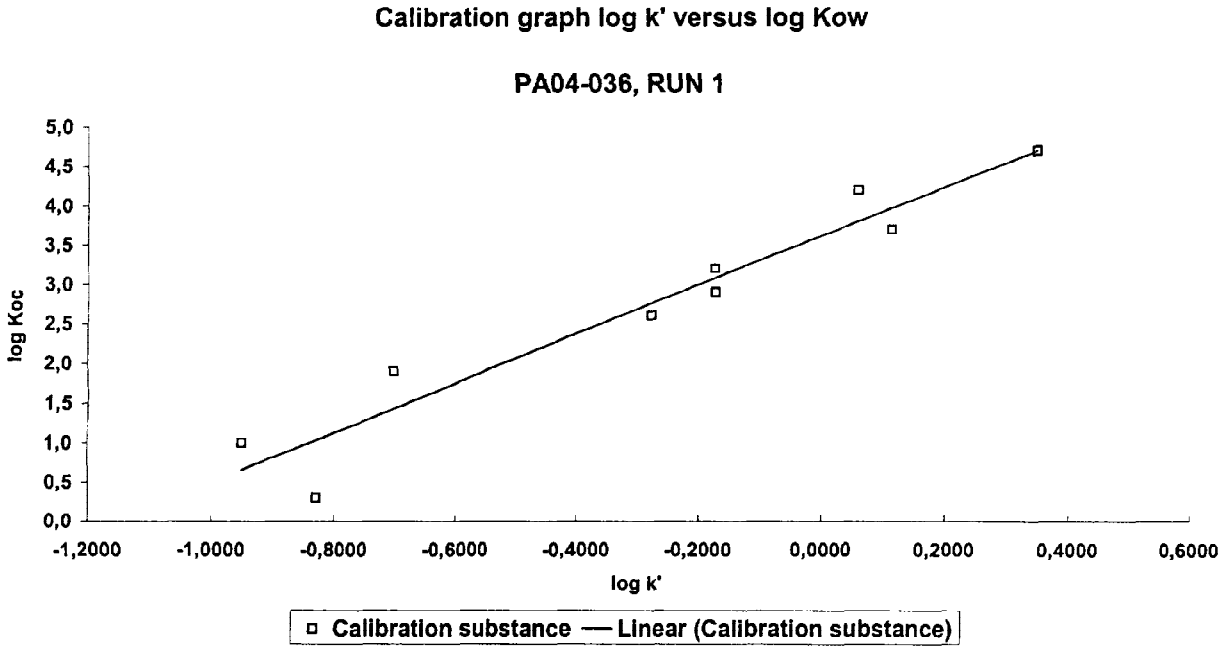
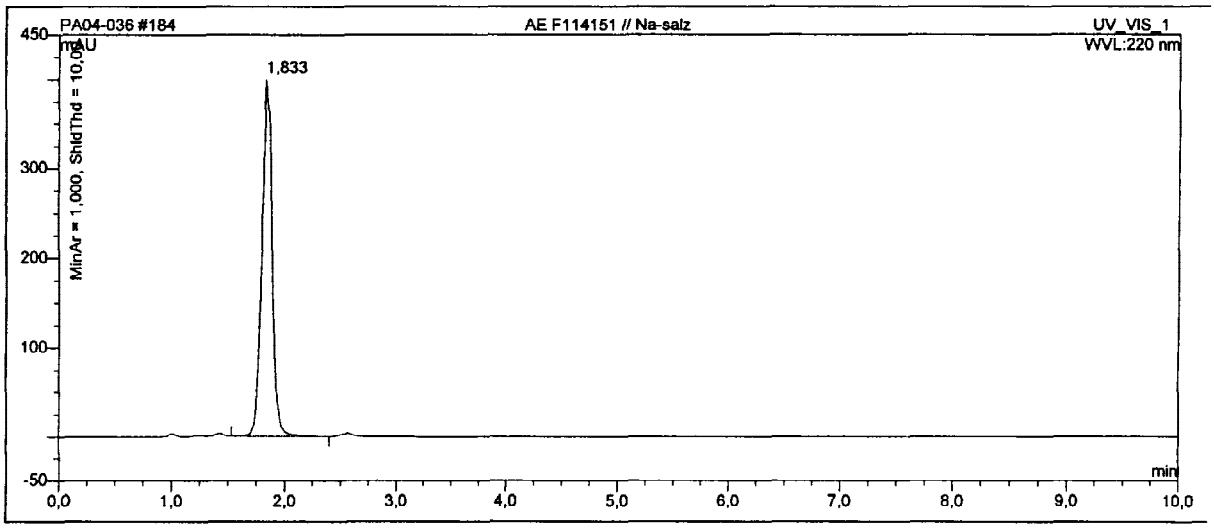


Figure 5: Typical chromatogram of the test item solution AE F114151 (acid conditions)

Sample Injection Report (incl. all peaks)

Sequence Name:	PA04-036
Directory:	CM_Oracle\AS_4\TEMPLATES\Sequences\AE F002671\PA04-036
Modification Time:	n.a. *Modification Operator:*

Sample Name:	**AE F114151 // Na-salz**	**Sample No.:**	184
Comment:	Einwaagen siehe Rohdaten vom 14.04.04	**Channel:**	UV_VIS_1
Sample Type:	unknown	**Injection vol.:**	20,0
Program Method:	PA04-036-LogPow-3	**Reference Inj. Vol.:**	n.a.
Quantitation Method:	AE F002671	**Dilution Factor:**	1,0000
Injection Time:	19.04.2004 20:42	**Sample Weight:**	1,0000
Position:	12	**ISTD Amount:**	1,0000



Integration Report

No.	Peak Name	Type	Ret.Time min	Height mAU	Area mAU*min	Rel.Area %
1	n.a.	BMB	1,83	399,143	41,928	100,00
Total:				**399,143**	**41,928**	**100,00**

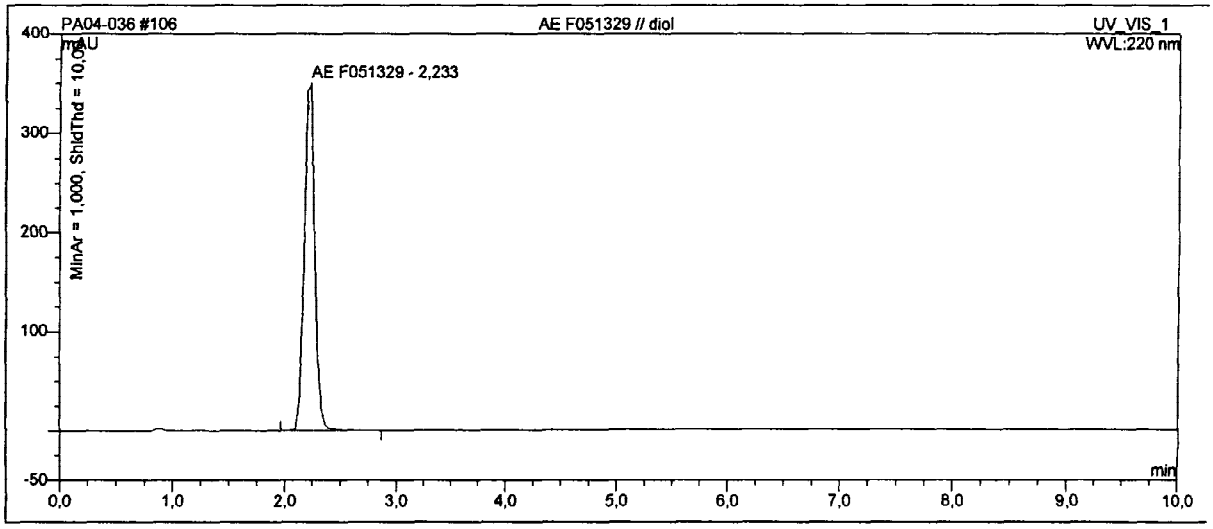
All Peaks within Signal Limit: **None**

Figure 6: Typical chromatogram of the test item solution AE F051329 (neutral conditions)

Sample Injection Report (incl. all peaks)

Sequence Name:	**PA04-036**
Directory:	**CM_Oracle\AS_4\TEMPLATES\Sequences\AE F002671\PA04-036**
Modification Time:	**n.a.** ***Modification Operator:***

Sample Name:	**AE F051329 // diol**	**Sample No.:**	106
Comment:	Einwaagen siehe Rohdaten vom 14.04.04	**Channel:**	UV_VIS_1
Sample Type:	unknown	**Injection vol.:**	20,0
Program Method:	PA04-036-LogPow-2	**Reference Inj. Vol.:**	n.a.
Quantitation Method:	AE F002671	**Dilution Factor:**	1,0000
Injection Time:	15.04.2004 20:04	**Sample Weight:**	1,0000
Position:	13	**ISTD Amount:**	1,0000



Integration Report

No.	Peak Name	Type	Ret.Time min	Height mAU	Area mAU*min	Rel.Area %
1	AE F051329	BMB	2,23	348,683	38,470	100,00
Total:				**348,683**	**38,470**	**100,00**

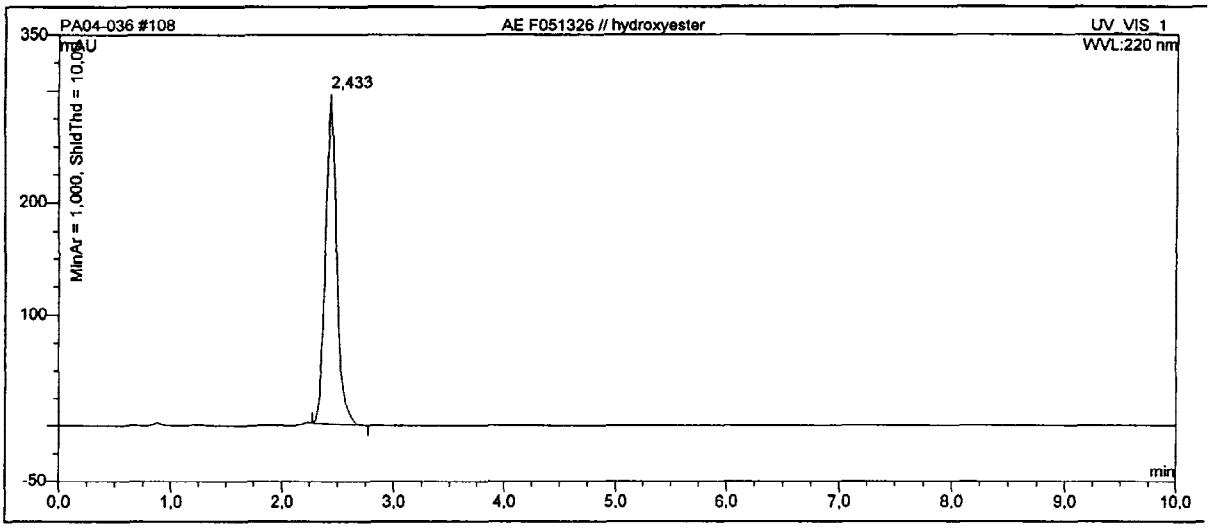
All Peaks within Signal Limit: **None**

Figure 7: Typical chromatogram of the test item solution AE F051326 (neutral conditions)

Sample Injection Report (incl. all peaks)

Sequence Name:	PA04-036
Directory:	CM_Oracle\AS_4\TEMPLATES\Sequences\AE F002671\PA04-036
Modification Time:	n.a. *Modification Operator:*

Sample Name:	**AE F051326 // hydroxyester**	**Sample No.:**	108
Comment:	Einwaagen siehe Rohdaten vom 14.04.04	**Channel:**	UV_VIS_1
Sample Type:	unknown	**Injection vol.:**	20,0
Program Method:	PA04-036-LogPow-2	**Reference Inj. Vol.:**	n.a.
Quantitation Method:	AE F002671	**Dilution Factor:**	1,0000
Injection Time:	15.04.2004 20:28	**Sample Weight:**	1,0000
Position:	14	**ISTD Amount:**	1,0000



Integration Report

No.	Peak Name	Type	Ret.Time min	Height mAU	Area mAU*min	Rel.Area %
1	n.a.	BMB	2,43	294,859	34,468	100,00
Total:				**294,859**	**34,468**	**100,00**

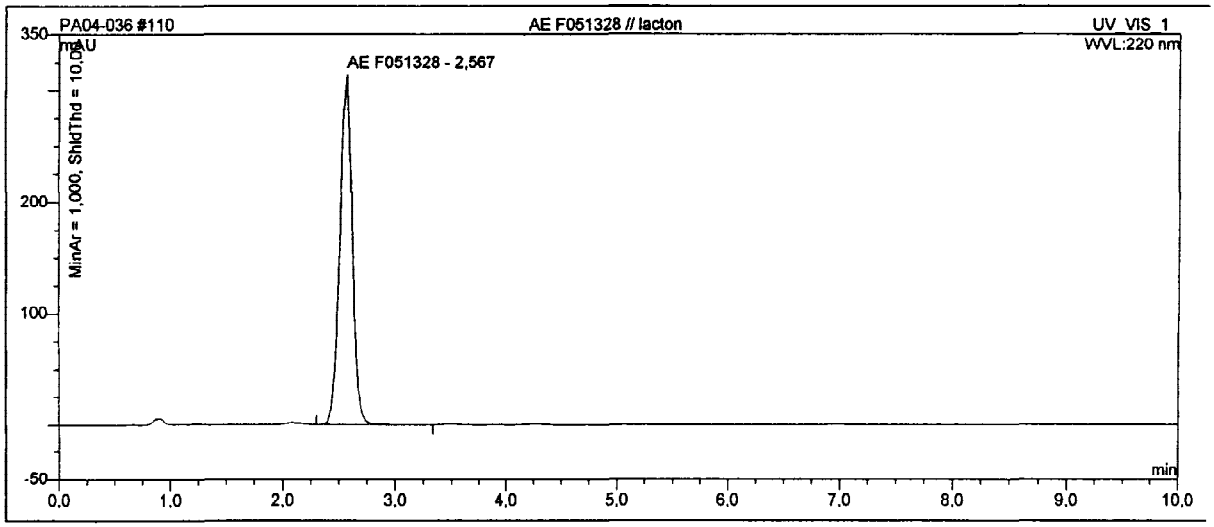
All Peaks within Signal Limit: **None**

Figure 8: Typical chromatogram of the test item solution AE F051328 (neutral conditions)

Sample Injection Report (incl. all peaks)

Sequence Name:	PA04-036
Directory:	CM_Oracle\AS_4\TEMPLATES\Sequences\AE F002671\PA04-036
Modification Time:	n.a. *Modification Operator:*

Sample Name:	**AE F051328 // lacton**	**Sample No.:**	110
Comment:	Einwaagen siehe Rohdaten vom 14.04.04	**Channel:**	UV_VIS_1
Sample Type:	unknown	**Injection vol.:**	20,0
Program Method:	PA04-036-LogPow-2	**Reference Inj. Vol.:**	n.a.
Quantitation Method:	AE F002671	**Dilution Factor:**	1,0000
Injection Time:	15.04.2004 20:52	**Sample Weight:**	1,0000
Position:	15	**ISTD Amount:**	1,0000



Integration Report

No.	Peak Name	Type	Ret.Time min	Height mAU	Area mAU*min	Rel.Area %
1	AE F051328	BMB	2,57	314,155	40,083	100,00
Total:				**314,155**	**40,083**	**100,00**

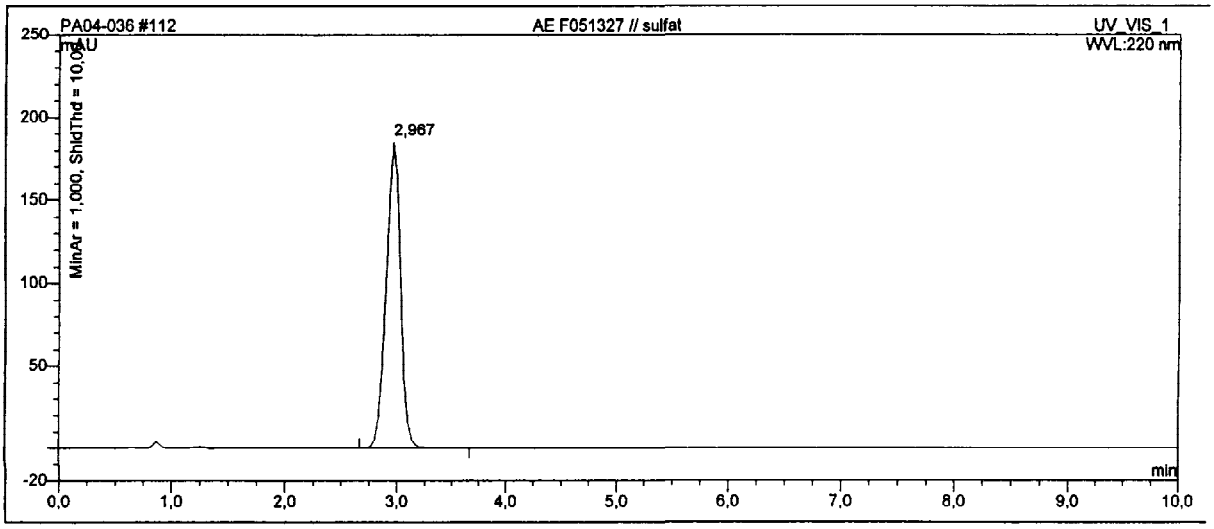
All Peaks within Signal Limit: **None**

Figure 9: Typical chromatogram of the test item solution AE F051327 (neutral conditions)

Sample Injection Report (incl. all peaks)

Sequence Name:	PA04-036
Directory:	CM_Oracle\AS_4\TEMPLATES\Sequences\AE F002671\PA04-036
Modification Time:	n.a. *Modification Operator:*

Sample Name:	**AE F051327 // sulfat**	**Sample No.:**	112
Comment:	Einwaagen siehe Rohdaten vom 14.04.04	**Channel:**	UV_VIS_1
Sample Type:	unknown	**Injection vol.:**	20,0
Program Method:	PA04-036-LogPow-2	**Reference Inj. Vol.:**	n.a.
Quantitation Method:	AE F002671	**Dilution Factor:**	1,0000
Injection Time:	15.04.2004 21:16	**Sample Weight:**	1,0000
Position:	16	**ISTD Amount:**	1,0000



Integration Report

No.	Peak Name	Type	Ret.Time min	Height mAU	Area mAU*min	Rel.Area %
1	n.a.	BMB	2,97	185,109	28,082	100,00
Total:				**185,109**	**28,082**	**100,00**

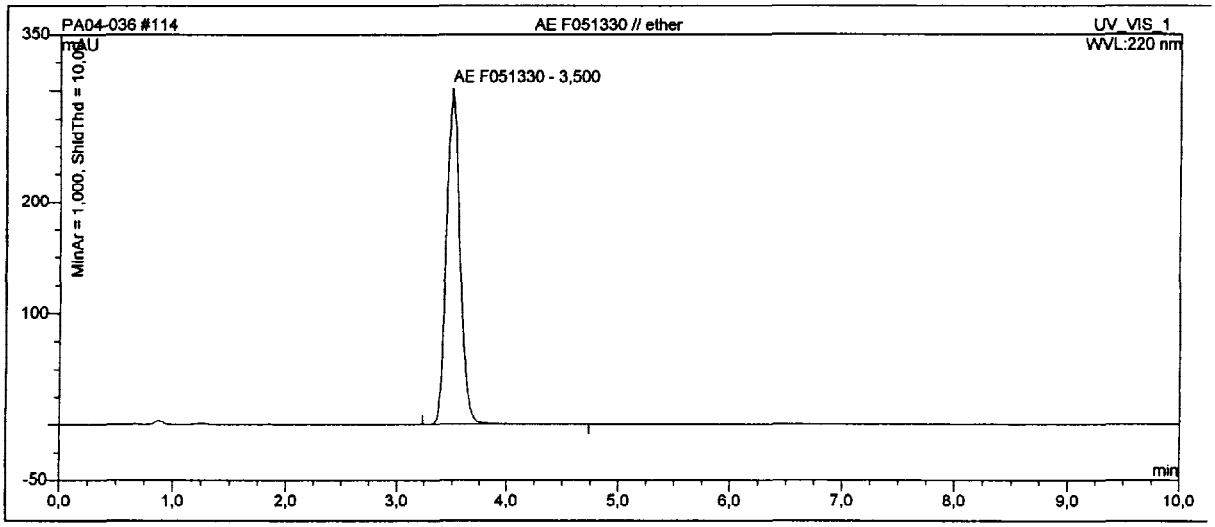
All Peaks within Signal Limit: **None**

Figure 10: Typical chromatogram of the test item solution AE F051330 (neutral conditions)

Sample Injection Report (incl. all peaks)

Sequence Name:	PA04-036
Directory:	CM_Oracle\AS_4\TEMPLATES\Sequences\AE F002671\PA04-036
Modification Time:	n.a. *Modification Operator:*

Sample Name:	**AE F051330 // ether**	**Sample No.:**	114
Comment:	Einwaagen siehe Rohdaten vom 14.04.04	**Channel:**	UV_VIS_1
Sample Type:	unknown	**Injection vol.:**	20,0
Program Method:	PA04-036-LogPow-2	**Reference Inj. Vol.:**	n.a.
Quantitation Method:	AE F002671	**Dilution Factor:**	1,0000
Injection Time:	15.04.2004 21:40	**Sample Weight:**	1,0000
Position:	17	**ISTD Amount:**	1,0000



Integration Report

No.	Peak Name	Type	Ret.Time min	Height mAU	Area mAU*min	Rel.Area %
1	AE F051330	BMB	3,50	301,608	42,828	100,00
Total:				**301,608**	**42,828**	**100,00**

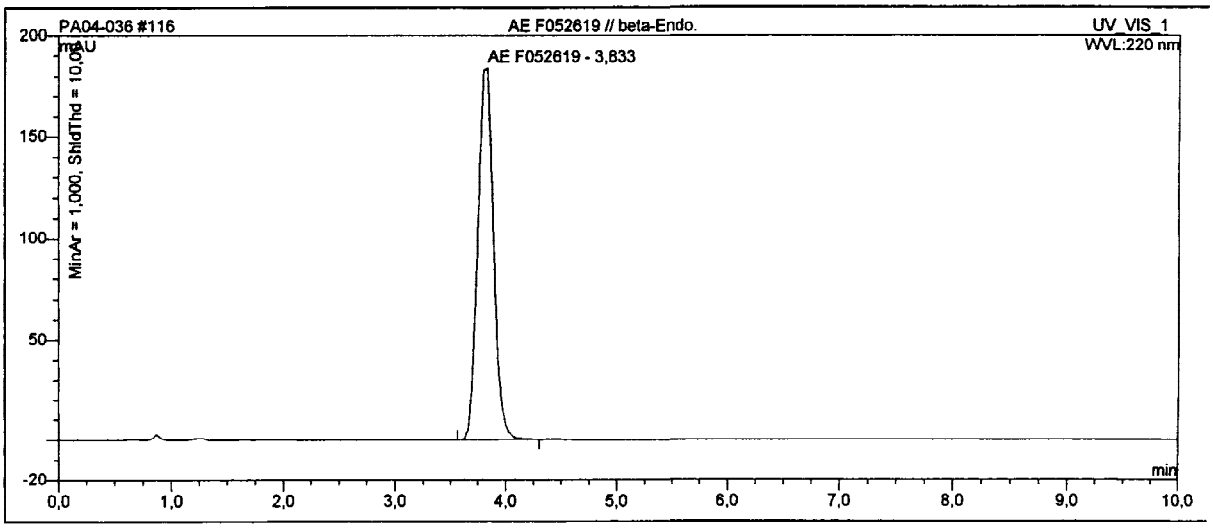
All Peaks within Signal Limit: **None**

Figure 11: Typical chromatogram of the test item solution AE F052619 (neutral conditions)

Sample Injection Report (incl. all peaks)

Sequence Name:	PA04-036
Directory:	CM_Oracle\AS_4\TEMPLATES\Sequences\AE F002671\PA04-036
Modification Time:	n.a. *Modification Operator:*

Sample Name:	**AE F052619 // beta-Endo.**	**Sample No.:**	116
Comment:	Einwaagen siehe Rohdaten vom 14.04.04	**Channel:**	UV_VIS_1
Sample Type:	unknown	**Injection vol.:**	20,0
Program Method:	PA04-036-LogPow-2	**Reference Inj. Vol.:**	n.a.
Quantitation Method:	AE F002671	**Dilution Factor:**	1,0000
Injection Time:	15.04.2004 22:04	**Sample Weight:**	1,0000
Position:	18	**ISTD Amount:**	1,0000



Integration Report

No.	Peak Name	Type	Ret.Time min	Height mAU	Area mAU*min	Rel.Area %
1	AE F052619	BMB	3,83	183,532	29,880	100,00
Total:				**183,532**	**29,880**	**100,00**

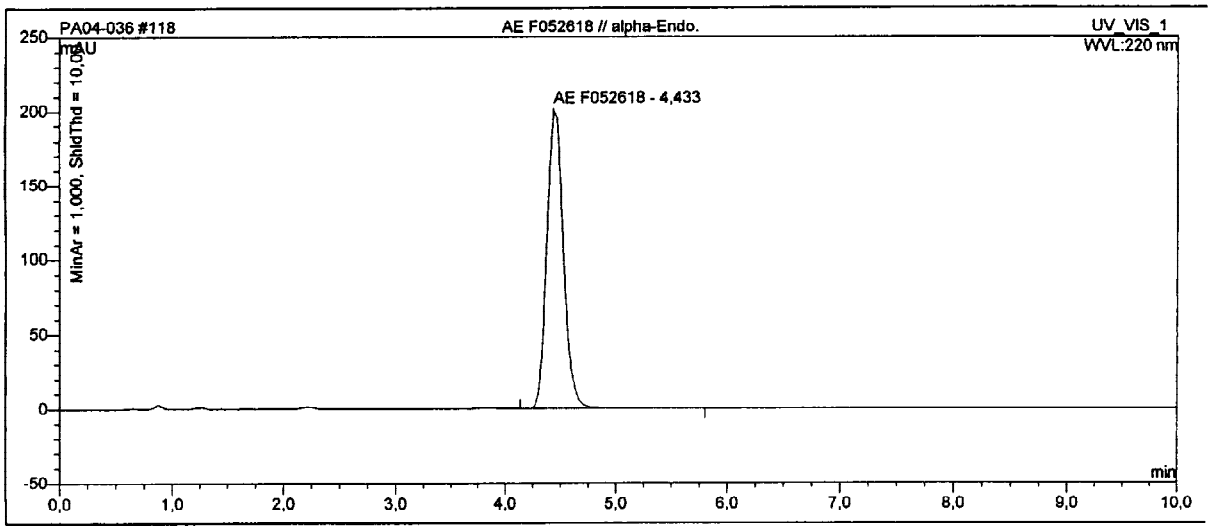
All Peaks within Signal Limit: **None**

Figure 12: Typical chromatogram of the test item solution AE F052618 (neutral conditions)

Sample Injection Report (incl. all peaks)

Sequence Name:	**PA04-036**
Directory:	**CM_Oracle\AS_4\TEMPLATES\Sequences\AE F002671\PA04-036**
Modification Time:	**n.a.** ***Modification Operator:***

Sample Name:	**AE F052618 // alpha-Endo.**	**Sample No.:**	118
Comment:	Einwaagen siehe Rohdaten vom 14.04.04	**Channel:**	UV_VIS_1
Sample Type:	unknown	**Injection vol.:**	20,0
Program Method:	PA04-036-LogPow-2	**Reference Inj. Vol.:**	n.a.
Quantitation Method:	AE F002671	**Dilution Factor:**	1,0000
Injection Time:	15.04.2004 22:28	**Sample Weight:**	1,0000
Position:	19	**ISTD Amount:**	1,0000



Integration Report

No.	Peak Name	Type	Ret.Time min	Height mAU	Area mAU*min	Rel.Area %
1	AE F052618	BMB	4,43	200,801	34,742	100,00
Total:				**200,801**	**34,742**	**100,00**

All Peaks within Signal Limit: **None**

7. GLP-Certificate

HESSISCHES MINISTERIUM
FÜR UMWELT, LANDWIRTSCHAFT
UND FORSTEN

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung/Test facillity ☐ Prüfstandort/Test site

Product Technology-Analytics Frankfurt
Bayer CropScience GmbH
Industriepark Höchst
65926 Frankfurt am Main

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and adress)

Prüfungen nach Kategorien/Areas of Expertise
(gemäß/according chemVwV-GLP Nr. 5.3/OECD guidance)

1 Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen

1 Physical and chemical properties and determination of content

22.10. und 23.10.2001
Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP- Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Im Auftrag

Th. Zimmermann, Referent, Wiesbaden, den 31. Juli 2002
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)

HESSISCHES MINISTERIUM FÜR UMWELT, LANDWIRTSCHAFT UND FORSTEN

Hess. Ministerium für Umwelt, Landwirtschaft und Forsten, Mainzer Straße 80 D65189 Wiesbaden
(Name und Adresse der GLP-Überwachungsbehörde/Name and address of the GLP Monitoring Authority)