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Development and Validation of HPLC-methods for the official control of Coccidiostatics and Antibiotics used as Feed AdditiveS (SMT4-CT98-2216)

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CANFAS - Collaborative study for the determination of nicarbazin in feedingstuffs and premixtures by HPLC

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ERRATUM

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Section 3.1.2 Sample homogeneity

For all feeds and the premixture the 'CV (between samples)' of the homogeneity test was calculated erroneously. The correct CV (between samples) has to be calculated by multiplying with the result of square root of 2. Table 3 must be as follows:

Table 3: Results of homogeneity tests for nicarbazin in broiler feeds and premixture

Results / Product	Declared content (mg/kg)	Measured content (mg/kg)	Homogeneity results	
			Between sample CV (%)	Within sample CV (%)
Broiler feed I	20	22,4	9,7	8,3
Broiler feed II	45	47,4	7,5	3,4
Broiler feed II	110	122	7,0	4,9
Broiler feed I	240	264	3,3	4,3
Premixture	1,10 %	1,15 %	6,1	4,8

For the 45, 110 and 240 ppm of nicarbazin containing feed and for the premixture the correction of CV's (between samples) does not influence the conclusion drawn about the homogeneity.
For the 20 ppm of nicarbazin containing feed the corrected CV (between samples) (9.7%) is higher than the maximum limit of 8%. The results of the collaborative study (Horrat-ratio of 1.22; see table 5) show that the sample was suitable. The rsd_r-value of 10.2% (see table 7) can be explained by the high value for CV (between samples).

SUMMARY

This report describes the results of a collaborative study of an HPLC method for the coccidiostat nicarbazin in five broiler feeds and one premixture. The collaborative study forms part of the EU-project "Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (CANFAS-SMT4-CT98-2216).

The principle of the method is as follows: samples are extracted by heating in a waterbath, mechanical shaking and sonoration using an acetonitrile/methanol mixture. For feedingstuffs, also water is added. The mixture is transferred in a volumetric flask. After settlement of the solids, an aliquot is filtered and assayed using a reverse-phase isocratic method, which measures the 4,4'dinitrocarbanilide (DNC) moiety at a wavelength of 350 nm.

The samples that were prepared for the collaborative study were 4 broiler feeds with declared nicarbazin contents of 20, 45, 110 and 240 mg/kg, 1 blank pig feed and 1 premixture with declared content of 1,1 % nicarbazin. The feed samples were sent to the participants as blind duplicates, the premixture as a single sample. The participants were asked to do a single determination per sample for the feed samples and to analyse the premixture in duplicate.

Results were reported by 19 laboratories. Statistical evaluation was performed according to ISO 5725.

The results of the collaborative study were evaluated in a meeting attended by the participants. The panel has accepted the results of the statistical evaluation, as described in par. 5.1, Table 7. Consequently it can be concluded that for feedingstuffs the repeatability and reproducibility of the method is acceptable. The results obtained for the recovery and for the blind blank samples are also satisfactory. The overall conclusion is that for feedingstuffs the performance of the method is satisfactory.

For the premixture the rsd_R (11,7 %) of the method is too high. According to the panel, a value of approx. 7 % should be attainable. It was decided that a new small-scale collaborative study will be organised (ca. 10 laboratories) with a modified method.

1 INTRODUCTION

Within the framework of the EU-project "Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (CANFAS-SMT4-CT98-2216), a method was developed for nicarbazin. Nicarbazin is a coccidiostat which is registered for broiler feeds with contents ranging between 40 - 50 mg/kg or 100 - 125 mg/kg.
The method was developed and validated by RIKILT, Wageningen, The Netherlands (see report H.J. Keukens, Development of Liquid Chromatographic Methods for the Determination of Nicarbazin in concentrates, premixtures and broiler feed, 01-11-1999). Subsequently, the method for feeds and premixtures was subjected to between-lab validation by the Universität Hamburg, Institut für Angewandte Botanik, Germany (see report H.-A. Putzka, 17-01-2000) and Istituto Superiore di Sanità, Roma, Italy (see report G. Brambilla, 26-01-2000) with satisfactory results (see Second Annual Report CANFAS, J. de Jong, 12-08-2000).
Prior to the collaborative study, a kick-off meeting was organised (Brussels, 13-14/6/2000) and participating laboratories were given the opportunity to familiarise themselves with the method, using feed samples with stated contents of nicarbazin. Also prior to the production of the materials for the collaborative study, separate batches of the materials were produced for stability testing, indicating that nicarbazin is stable at room temperature for 4 months.
The samples which were prepared for the collaborative study were 4 broiler feeds with declared nicarbazin contents of 20, 45, 110 and 240 mg/kg, 1 blank feed and 1 premixture with declared content of 1,1 % nicarbazin. The feeds with 20 and 240 mg nicarbazin per kg have been included in order to assure that the method is applicable for contents 2 times lower than the lowest permitted content resp. 2 times higher than the highest permitted content.
The feed samples were sent to the participants as blind duplicates, the premixture as a single sample. The participants were asked to do a single determination per sample for the feed samples and to analyse the premixture in duplicate. Before these samples were shipped, the between-sample homogeneity was checked with satisfactory results (see par. 3.1.2).
Together with the samples, a letter with instructions, reporting forms, etc. was sent to the participants (see Appendix 1).
This report describes the results of the collaborative study.

2 PARTICIPANTS

The following laboratories/persons participated in the collaborative study.

- CCL-Nutricontrol, Veghel, The Netherlands; R. Margry, J.G.P. van der Palen.
- IEEB, Bordeaux, France; J.P. Antalick, T. Gron.
- INETI/DTIA, Lisbon, Portugal; I. Felgueiras, C. Saldanha.
- Istituto Superiore di Sanita, Lab. Med. Veterinaria, Roma, Italy; G. Brambilla, C. Cartoni, M. Fiori.
- Istituto Zooprofilattico Sperimentale della Lombardia e dellémilia Ronagna, Reparto Chimico, Brescia, Italy; E. Faggionato, A. Baiguera.
- Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy; C. Testa, N. Rubattu, A. Serra, E. Azara.
- Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy; G. Biancotto, B. Allegretta, D. Berto, V. Capuzzo.
- Istituto Zooprofillatico Sperimentale delle regioni Lazio e Toscana, Roma, Italy; A. Ubaldi, A. di Lullo.
- Laboratorio Nacional de Sanidad y Produccion Animal - M.A.P.A., Santa Fe, Spain; R. Checa-Moreno, A. Ariza-Avidad.
- Laboratory of the Government Chemist, Teddington, United Kingdom; G. Merson, J. Cowles, K. Needham.
- LNIV, Lisbon, Portugal; J.M. Nunes da Costa, M.B. Casqueira.
- LUFA - Augustenberg, Karlsruhe, Germany; K. Michels, S. Witzemann.
- LUFA-ITL Kiel, Kiel, Germany; F.H. Johannsen, Kollwitz.
- Masterlab, Putten, The Netherlands; K. van Schalm, A. Schaaf.
- Pre-Mervo Kwaliteitsdienst, Utrecht, The Netherlands; C. Schreuder.
- Rijksontledingslaboratorium, Tervuren, Belgium; K. Haustraete, A. Fontaine, M. Bral, R. van San.
- RIKILT, Wageningen, The Netherlands; H.J. van der Kamp, E.A. Jansen.
- Universität Hamburg, Institut für Angewandte Botanik, Hamburg, Germany; H. Putzka, D. Böhm.
- Universität Hohenheim, Landesanstalt für Landwirtschaftliche Chemie, Stuttgart, Germany; B. Eckstein, K. Schwadorf, E. Koenzen.

3 MATERIALS

3.1 Samples for collaborative study

3.1.1 Sample composition

Specifications of the samples, which were produced for the collaborative study, are given in Table 1.

Table 1: Specifications of the samples

Type of feed / premixture	Declared content	Units	Subcontractor	Date of production
Broiler feed I, 6 % fat	20	mg/kg	IPC – Dier, Barneveld (NL)	07/09/2000
Broiler feed II, 11 % fat	45	mg/kg	IPC – Dier, Barneveld (NL)	07/09/2000
Broiler feed II, 11 % fat	110	mg/kg	IPC – Dier, Barneveld (NL)	08/09/2000
Broiler feed I, 6 % fat	240	mg/kg	IPC – Dier, Barneveld (NL)	08/09/2000
Premixture for broiler feed II	1,1	%	Trouw Nutrition, Putten (NL)	July 2000

The complete composition of the feeds is given in Appendix 2 (in Dutch). The premixture was based on inorganic support material and contained regular contents of vitamins, minerals and trace elements.

The main composition of the two feeds is given in Table 2.

Table 2: Main composition of the two feeds

Product / Ingredient	Broiler I	Broiler II
Crude protein (%)	21,6	21,2
Crude fat (%)	6,0	11,0
Starch (%)	35,1	30,7
Crude fibre (%)	2,7	3,1
Crude ash (%)	6,0	6,3
Moisture (%)	12,3	12,2

The composition of the feeds (I and II) and the premixture was the same as the composition of the products which were produced by IPC-Dier and Trouw in February 2000 for stability testing (see Report on homogeneity and stability studies of samples for the collaborative studies for nicarbazin, J.J.M. Driessen and J. de Jong, RIKILT, Wageningen, NL, 12/10/2000).

The feed products have been prepared in a quantity of 500 kg each. To achieve a maximum degree of homogeneity halfway through the production 36 kg of feed are withdrawn from the stream for subsampling activities and put into two sacks of 18 kg. After discarding the top layer (ca. 2 kg) about 30 - 50 subsamples of approx. 250 grams have been taken (manual distribution with a shovel) from each of these sacks. The subsamples were stored in double paper sacks. From the premixture 60 subsamples of approx. 80 g have been taken by means of an automatic subsampling device and supplied in plastic bottles.
All subsamples have been stored at room temperature (ca. 20 °C).
Next to the above mentioned samples which contained nicarbazin, a blind blank feed was sent to the participants as well as a blank feed labelled "blank feed for nicarbazin recovery purposes" (see Appendix 1). The blind blank feed was a pig feed containing carbadox and olaquindox (a mixture of 2 pig feeds produced for the CANFAS collaborative studies for carbadox and olaquindox, see the corresponding reports). This feed was analysed at RIKILT prior to the collaborative studies and was found to contain no detectable amounts of nicarbazin or interfering substances. The blank feed for nicarbazin recovery purpose was a broiler feed containing 2 mg/kg virginiamycin, produced for the CANFAS collaborative study (see RIKILT report 2002.017). This feed was also analysed prior to the collaborative study and contained 3 mg/kg nicarbazin, probably due to carry-over during production.

3.1.2 Sample homogeneity

The homogeneity of the samples was studied by RIKILT by random selection of 10 subsamples, applying the HPLC-method developed in CANFAS (see Annex 1 of Appendix 1).
The results of the homogeneity determinations of the individual feeds / premixture are attached in Appendix 3. Table 3 gives a summary of these results.

Table 3: Results of homogeneity tests for nicarbazin in broiler feeds and premixture

Results / Product	Declared content (mg/kg)	Measured content (mg/kg)	Homogeneity results	
			Between sample CV (%)	Within sample CV (%)
Broiler feed I	20	22,4	6,8	8,3
Broiler feed II	45	47,4	5,3	3,4
Broiler feed II	110	122	5,0	4,9
Broiler feed I	240	264	2,4	4,3
Premixture	1,10 %	1,15 %	4,3	4,8

According to the Project Plan the CV's for homogeneity should not exceed 2 times the CV's for repeatability ($CV_{hom} \leq 2\ CV_r$). Based on previous results of within-lab validation (see Second Annual Report CANFAS, J. de Jong, 12-08-2000) the maximum limit for CV_{hom} was set to 8 % for the feeds and 7 % for the premixture.
All between-sample CV's fulfil these requirements while only the within-sample CV for the 20 ppm feed slightly exceeds 8 %. Thus, it is concluded that the samples are sufficiently homogeneous.

3.1.3 Sample logistics

The feed samples were sent as blind duplicates. The codes are given in Appendix 4. The premixture was sent as a single sample and was labelled as such. The samples were sent to the participants by courier service on 2 October 2000 together with a letter with instructions (Appendix 1). During transport no special precautions were taken with regards to the temperature of the samples.

3.2 Reference standard

The reference standard was supplied by mr. D. Towell, Eli Lilly, Liverpool (UK). The specifications of the reference standard (Lot Nr. X47623) are described in Appendix 5. The participants were instructed to set the purity of the reference standard to 100 % (see Appendix 1).

4 METHODS

4.1 Method of analysis

The method of analysis is included as annex 1 to Appendix 1. The participants were instructed that this method has to be used without any modifications.

4.1.1. HPLC-conditions

Various types of HPLC-columns were used (the column which is recommended in the method is a Nova-Pak C-18 300 x 3,9 mm with a particle size between 5 and 10 µm).
The mobile phase described in the method is Acetonitrile-Water 65/35. Three laboratories used a different mobile phase.
The HPLC conditions (Column and mobile phase) used by the participants are shown in Table 4.

4.2 Method for statistical evaluation

Statistical evaluation was performed according to ISO 5725 Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method (First edition, 1994-12-15).
The scrutinity of results for consistency and outliers was checked by

a) Graphical consistency techniques: Mandel's h plot for between-laboratory variability, Mandel's k plot for within-laboratory variability
b) Numerical outlier techniques: Cochran's test of the within-laboratory variability, Grubbs' test (single and double) for between-laboratory variability

Whenever necessary and appropriate, laboratories which showed consistently high within-cell variation and/or extreme cell means across many levels and/or Cochran or Grubbs' outliers were contacted to try to ascertain the cause of the discrepant behaviour.
The Horwitz equation and the HORRAT ratios form the basis for the evaluation of the reproducibility of the method. The HORRAT ratios are given in Table 5. The HORRAT ratios should be lower than 2 (see W. Horwitz and R. Albert, J.A.O.A.C. 74 (1991) 718-744).

Table 4: HPLC-conditions

Partner	Column	Mobile phase
13	As described in the method.	As described in the method.
15	Hypersil ODS (5 µm) 200 x 4,6 mm + Guard column	Water 50 %/ Acetonitrile 50%
16	Spherisorb ODS 1, (5 µm) 250 x 4 mm	As described in the method.
19	250/4,6 Nucleosil 120 - 5 C18	As described in the method.
20	Alltech Alltime C18 250 x 4,6 (5 µm)	As described in the method.
21	Supelcosil LC18 25 cm x 4,6 mm (5 µm) with Supelguard LC18	As described in the method.
23	Not reported	Not reported
24	250 mm x 4,6 mm C18 (5 µm)	As described in the method.
25	As described in the method	As described in the method.
26	Luna ODS-2 250 x 4,6 mm	As described in the method.
27	As described in the method; but according to the catalogue of the firm Waters the particle size of the reacted silica in Nova-Pak columns is always 4 µm in diameter	As described in the method.
29	Nova Pak, 250 x 4,6 mm; C18 (4µm)	As described in the method.
30	As described in the method	As described in the method.
31	Bondapak C18 300mm x 3,9 mm ID	As described in the method.
32	Waters Symmetry, C18 (5µm) 4,6 mm x 250 mm (Part no. WAT 054215)	As described in the method.
33	C18 ODS	Ammoniumacetatebuffer 4,8 / acetonitrile = 55/45
35	Lichrospher 100-RP18 (5 µm) 125 x 4,0 mm	Methanol/Water = 700/300
38	Hypersil ODS C18, 250 x 4,6 mm (5 µm)	As described in the method.
39	125 x 3 mm, Lichrospher RP18 (5 µm) Merck 51232	As described in the method.

5 RESULTS

For each participant the reported results for the samples, the completed questionnaire and representative chromatograms are annexed in Appendix 6.

5.1 Statistical evaluation

The results reported by the participants are given in Table 6. Figure 1 demonstrates the Mandel h and k plots of these results. From the Mandel h plot it can be concluded that the results of laboratories 20, 32 and 38 show extreme cell means across many levels.
The following laboratories were contacted to try to ascertain the cause of the discrepant behaviour:

- Lab 19 about the Cochran outlier for the 20 mg/kg sample: no cause could be found.
- Lab 20 about the extreme values at all levels: the cause was identified: the results were calculated with a calibration curve prepared from an incompletely dissolved stock standard solution in extraction solvent. The results were recalculated with a calibration curve prepared from a stock standard solution in DMF which was run in the same series as the sample extracts. The new results are given in Table 7.
- Lab 25 about the Cochran outlier for the 240 mg/kg sample: the outlier was probably caused by a technical problem (malfunctioning of the autosampler).
- Lab 26 about the Grubbs upper outlier (after eliminating lab 20) for the 240 mg/kg sample: no cause could be found.
- Lab 32 about the extreme values at all levels: the cause was identified: the dilution of the samples (see par. 6.2.1 and 6.2.2) was done with extraction solvent in stead of with HPLC eluent. The laboratory reanalysed the samples; the new results are given in Table 7.
- Lab 38 about the extreme values at many levels as well as the low results for its recovery experiments (83%): this laboratory replied that "due to their material requirements as centrifuge and other disposable flask, they weighed 1,25 g of sample feed and diluted to 50 ml". However, this is not regarded as a cause for the extreme values at many levels; no other causes could be found.

Based on the findings mentioned above it was decided to replace the results of laboratories 20 and 32 by the new results, see Table 7. The corresponding Mandel h and k plots are shown in Figure 2.
The Cochran outliers (labs 19 and 25: for the 20 mg/kg sample and the 240 mg/kg sample respectively) and the Grubb's upper outlier lab 26 for the 240 mg/kg sample were discarded. Moreover, all the results of lab 38 were discarded for the following reasons: the recovery of lab 38 (83 %) is significantly lower (Grubbs outlier) than the mean recoveries of the other laboratories which are all higher than 90 % (see par. 5.3). This is in line with the results obtained in the method development and between-lab validation phases of the CANFAS project where recoveries were consistently higher than 90 % (see Second Annual Report CANFAS, J. de Jong, 12-08-2000). The low results that were obtained by lab 38 for all samples (except for the premixture) can be

explained by the low recovery. This means that the method is not under control in this lab and the results should not be taken into account in the statistical evaluation.
The resulting Mandel h and k plots are shown in Figure 3. The resulting values for the statistical parameters (mean, relative standard deviations for repeatability and reproducibility) are given in Table 7.
According to the Project Plan, the rsd_r-values should be ≤ 10 %. Only for the 20 mg/kg sample this value is slightly higher and consequently the conclusion seems to be justified that the repeatability is acceptable.
For low contents, the repeatability could be improved by increasing the weight to 10 g (see remark 9.1 of the method).
The Horwitz equation and the HORRAT ratios form the basis for the evaluation of the reproducibility (see W. Horwitz and R. Albert, J.A.O.A.C. 74 (1991) 718-744). The HORRAT ratios are given in Table 5. The HORRAT ratios should be lower than 2. For the four feed samples this criterion is met and established rsd_R-values are in line with values predicted by the Horwitz equation. For the premixture, the HORRAT ratio is too high. In the evaluation meeting it was discussed how to proceed. The following options were regarded:

- Redo the collaborative study for the premixture after modifications in the method
- Conclude that the method is not suitable for premixtures
- Accept the relatively high CV_R for the premixture

The results of the discussion in the evaluation meeting are described in Chapter 6 of this report.

Table 5: Horrat ratios of the Nicarbazin collaborative study

Mean after elimination of outliers[1] (mg/kg)	Predicted rsd_R	Established rsd_R	Horrat[2]	Conclusion
22,3	10,024	12,260	1,22	Reproducibility OK
45,1	9,019	9,060	1,00	Reproducibility OK
117	7,824	7,918	1,01	Reproducibility OK
260	6,929	4,843	0,70	Reproducibility OK
11.247	3,933	11,660	2,97	Reproducibility not OK

[1] = lab 19/sample 20 mg/kg; lab 25 and 26/sample 240 mg/kg; lab 38 all results
[2] = Horrat is the ratio between the established rsd_R and the predicted rsd_R

Table 6. Nicarbazin in four broiler feeds and one premixture for broiler feed

(with first results of labs 20 and 32)

	Result (mg/kg)									
Sample	NIC 20 mg/kg		NIC 45 mg/kg		NIC 110 mg/kg		NIC 240 mg/kg		NIC 11000 mg/kg	
Lab										
13	23	21	46	46	128	124	275	270	10292	10933
15	28	23	45	48	118	124	269	287	10640	10970
16	18	19	45	46	113	105	245	245	10345	10018
19	*21*$^{Co/Gduo}$	*44*$^{Co/Gduo}$	46	45	115	120	260	270	11450	10870
20	*42*$^{Guo/Gduo}$	*43*$^{Guo/Gduo}$	*76*$^{Guo/Gduo}$	*80*$^{Guo/Gduo}$	*160*^{Gus}	*160*^{Gus}	*351*^{Gdus}	*361*^{Gdus}	*21297*$^{Guo/Gduo}$	*21633*$^{Guo/Gduo}$
21	22	21	52	45	119	122	252	252	9342	9461
23	30	21	44	40	100	118	241	261	10600	9900
24	23	23	48	50	112	109	265	265	12728	12968
25	20	20	36	36	100	100	*286*^{Co}	*210*^{Co}	*13900*^{Gduo}	*13900*^{Gduo}
26	24	20	*53*^{Gduo}	*45*^{Gduo}	115	128	342^{Gdus}	359^{Gdus}	11833^{Cs}	13462^{Cs}
27	20	20	43	44	115	119	253	261	11698	11458
29	21	23	46	46	127	126	248	254	11220	10199
30	24	20	38	48	146	120	261	274	11920	12000
31	21	23	49	46	117	115	266	270	10200	11010
32	*14/13*	*16/12*	*33/34*	*34/33*	*80/83*	*88/83*	*180/180*	*182/178*	*7642*	*7643*
33	22	20	46	42	108	114	236	232	10260	10550
35	24	23	48	41	103	123	260	244	10930	11484
38	15	18	42	36	93	93	180	182	10242	10336
39	21	17	44	43	114	117	259	264	10194	10453

number of labs	16	16	17	16	16
m (mg/kg)	21	44	115	259	10936
rsd_r (%)	11,1	7,1	6,3	2,8	4,1
rsd_R (%)	13,5	9,1	9,5	12,9	9,0

Remark 1: *laboratory 32 analyzed each feed sample in duplicate; all samples were analyzed after unproper extraction*

Remark 2: Italic printed results are not taken into account in the statistical evaluation!

Key to symbols:

result^{Co} = Cochran outlier

result^{Cs} = Cochran straggler

result^{Guo} = Grubb's upper outlier

result^{Gduo} = Grubb's double upper outlier

result^{Gus} = Grubb's upper straggler

result^{Gdus} = Grubb's double upper straggler

Table 6: Nicarbazin in four broiler feeds and one premixture for broiler feed (with first results of labs 20 and 32)

Table 7. Nicarbazin in four broiler feeds and one premixture for broiler feed
(with new results from labs 20 and 32)

	Result (mg/kg)									
Sample	NIC 20 mg/kg		NIC 45 mg/kg		NIC 110 mg/kg		NIC 240 mg/kg		NIC 11000 mg/kg	
Lab										
13	23	21	46	46	128	124	275	270	10292	10933
15	28	23	45	48	118	124	269	287	10640	10970
16	18	19	45	46	113	105	245	245	10345	10018
19	*21* $^{Co/Gus/Gdus}$	*44* $^{Co/Gus/Gdus}$	46	45	115	120	260	270	11450	10870
20	26 ^{Gdus}	27 ^{Gdus}	50	54	121	121	271	274	13693	13673
21	22	21	52	45	119	122	252	252	9342	9461
23	30	21	44	40	100	118	241	261	10600	9900
24	23	23	48	50	112	109	265	265	12728	12968
25	20	20	36	36	100	100	*286* ^{Co}	*210* ^{Co}	13900	13900
26	24	20	53	45	115	128	*342* ^{Guo}	*359* ^{Guo}	11833	13462
27	20	20	43	44	115	119	253	261	11698	11458
29	21	23	46	46	127	126	248	254	11220	10199
30	24	20	38	48	146	120	261	274	11920	12000
31	21	23	49	46	117	115	266	270	10200	11010
32	23/21	21/24	44/53	48/48	124/113	122/129	266/271	264/263	9932	10414
33	22	20	46	42	108	114	236	232	10260	10550
35	24	23	48	41	103	123	260	244	10930	11484
38	*15*	*18*	*42*	*36*	*93*	*93*	*180*	*182*	*10242*	*10336*
39	21	17	44	43	114	117	259	264	10194	10453

number of labs	17	18	18	16	18
m (mg/kg)	22,0	45,0	117	260	11247
rsd_r (%)	10,2	7,1	6,0	2,6	3,8
rsd_R (%)	12,3	9,1	7,9	4,8	11,7

Remark 1: laboratory 32 reported two replicates per feed sample; every second result has not been taken into account in the statistical evaluation!

Remark 2: Italic printed results are not taken into account in the statistical evaluation!

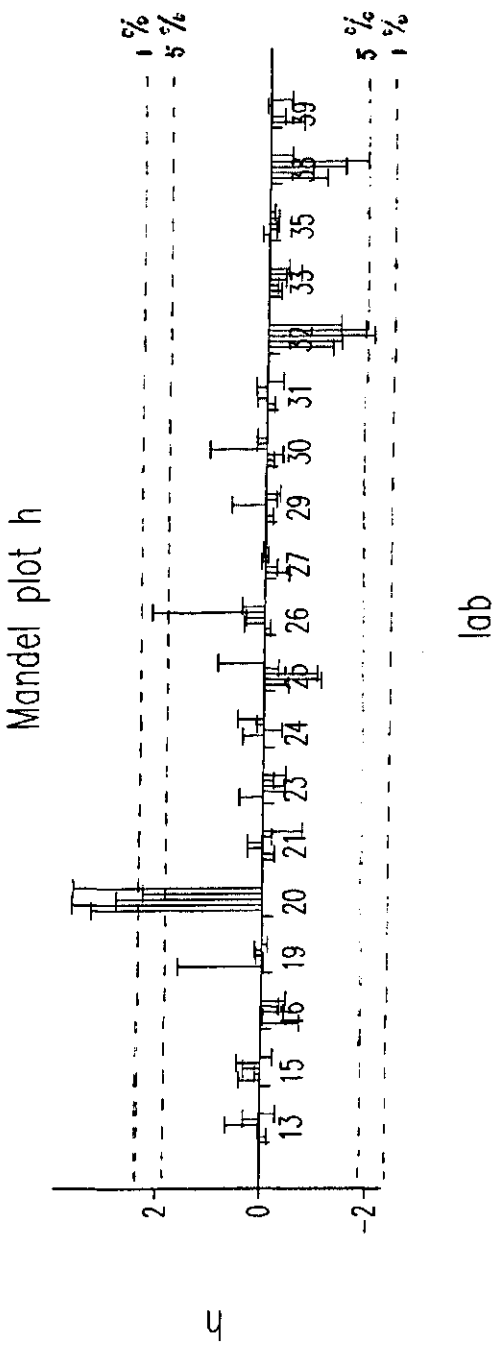
Key to symbols:

result^{Co} = Cochran outlier result^{Gus} = Grubb's upper straggler

result^{Guo} = Grubb's upper outlier result^{Gdus} = Grubb's double upper straggler

Table 7: Nicarbazin in four broiler feeds and one premixture for broiler feed (with new results of labs 20 and 32)

Figure 1: Mandel h and k plots of the results reported by the participants (with first results of labs 20 and 32)



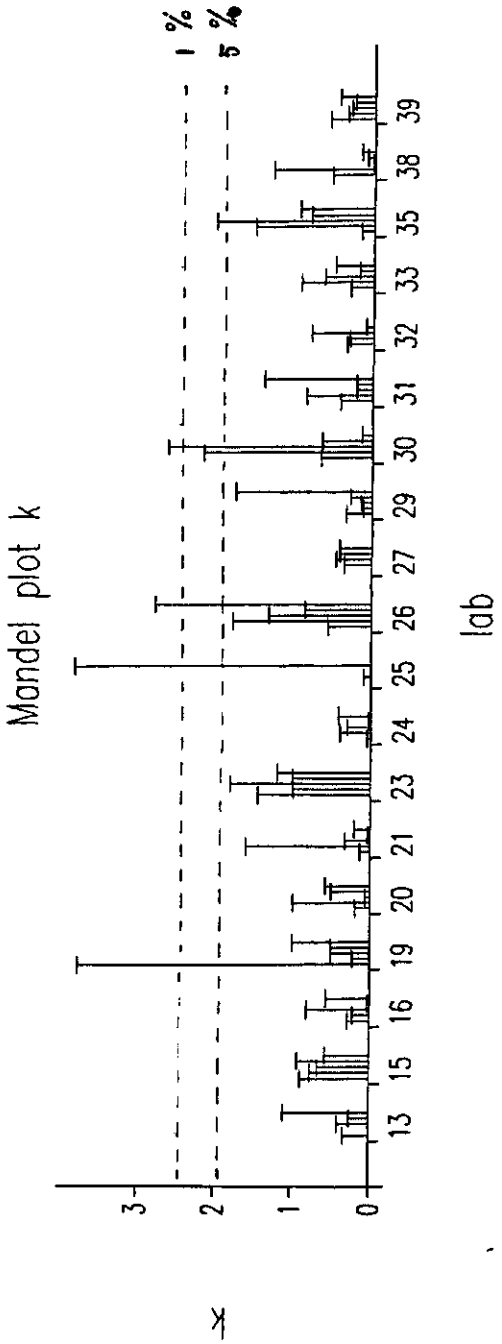


Figure 2: Mandel h and k plots with new results from labs 20 and 32

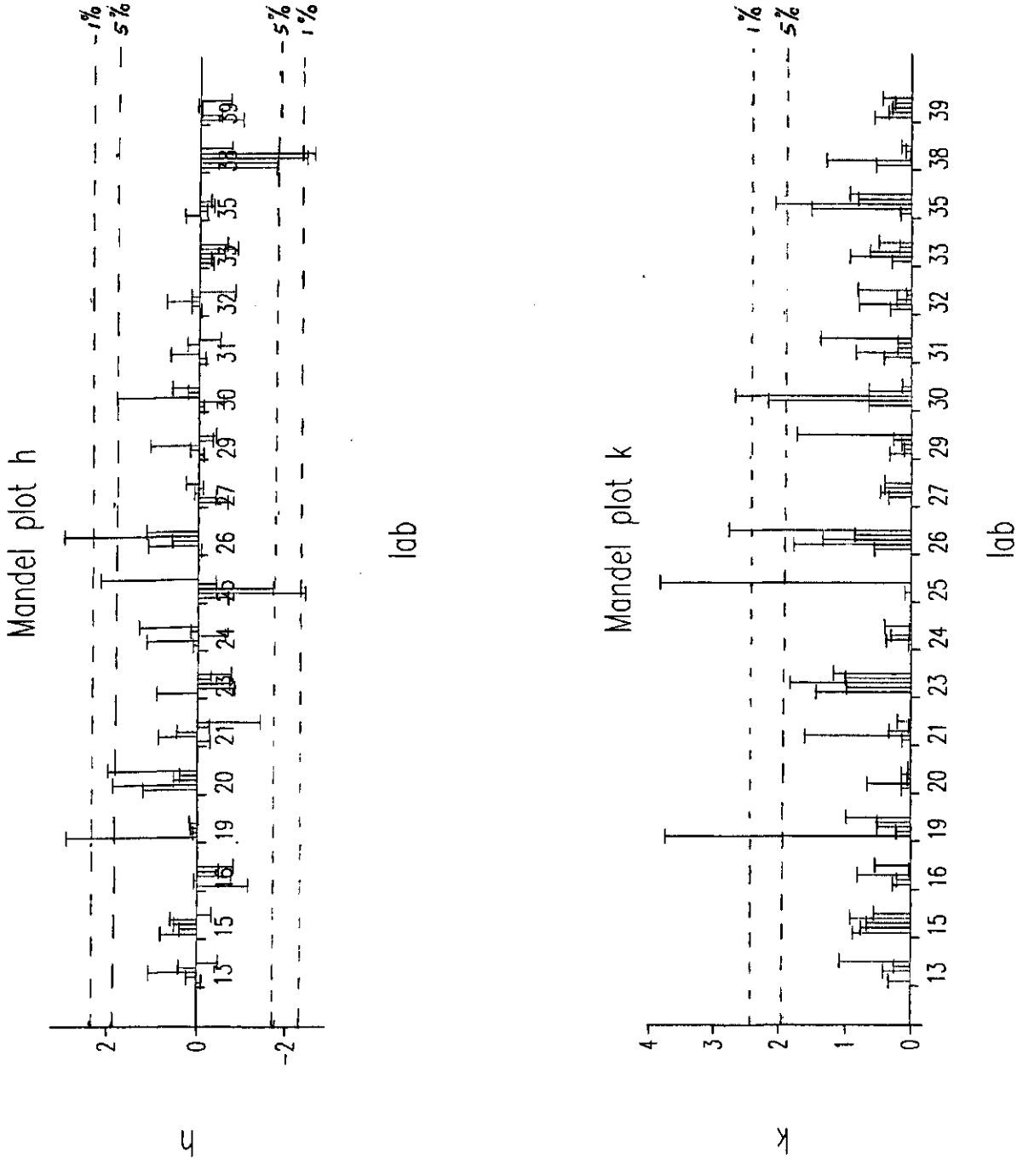
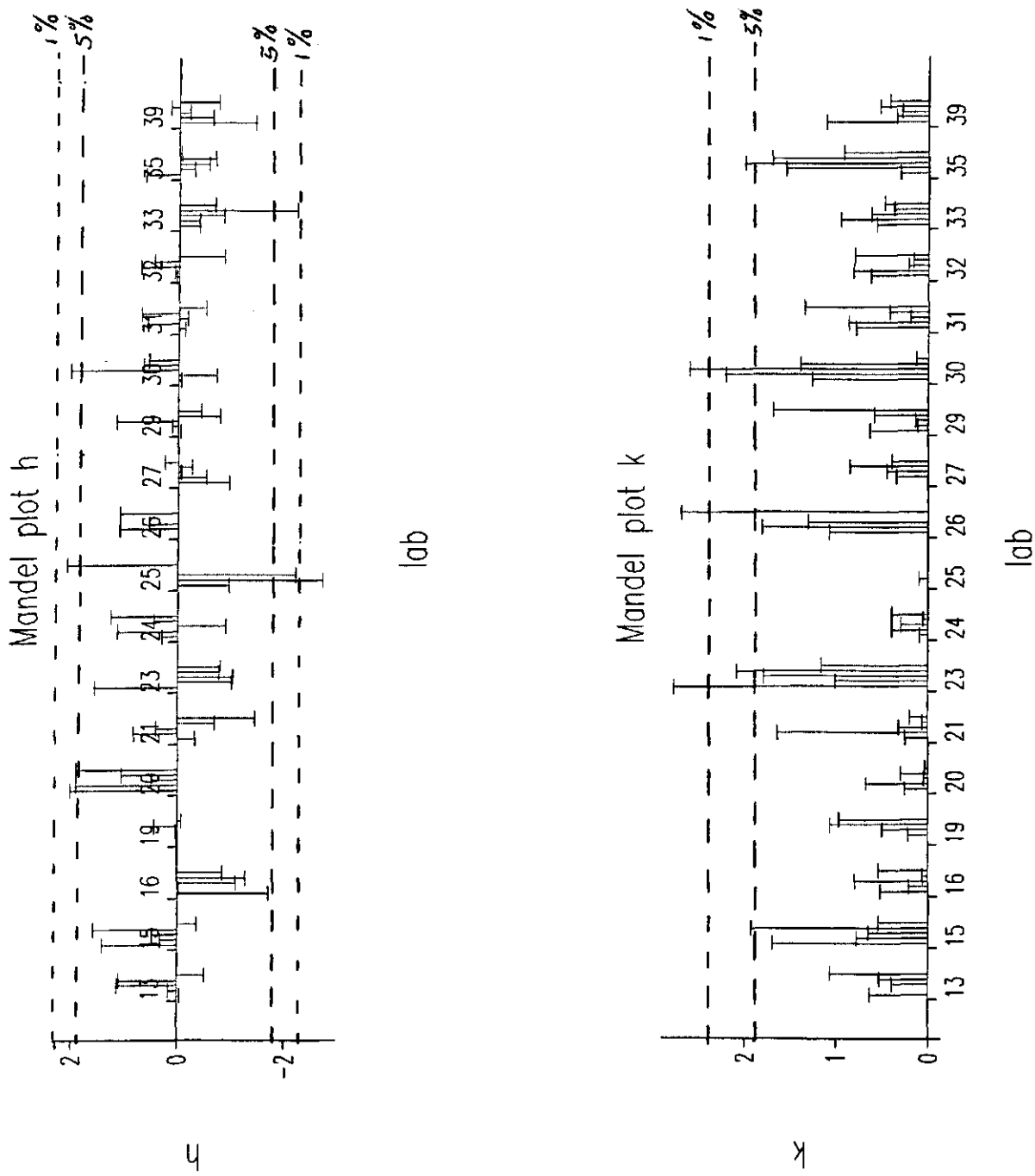


Figure 3: Mandel h and k plots after elimination of outliers (lab 19/ sample 20 mg/kg; lab 45 and lab 26/sample 240 mg/kg) and all results of lab 38



5.2 Blank samples

Table 8: Reported results of the participants for the blank samples

Partner	Blank sample 1	Blank sample 2
13	0 mg/kg	0 mg/kg
15	Blank	Blank
16	Not found	Not found
19	0,4	1,3
20	Neg	Neg
21	0,0 N.D.	0,0 N.D.
23	<5,93	<5,93
24	Blank	Blank
25	Nd	Nd
26	0	0
27	Not detectable	Not detectable
29	0	0
30	<3	<3
31	1,4	1,5
32	Negative	Negative
33	<2	<2
35	2	1
38	0	0
39	<2	<2

Three laboratories (19, 31 and 35) detected small contents of nicarbazin in the blank samples.

5.3 Recoveries

Table 9: Recoveries

Partner	Recovery 1 in %	recovery 2 in %	recovery average in %
13	97	98	98
15	99	99	99
16	101	102	102
19			101
20	108	108	108
21			100
23	Not reported		Not reported
24	99	101	100
25			91
26			101
27			100
29	88	103	95
30			99
31	101	99	100
32	100	96	98
33			99
35	99	99	99
38			83
39	102	102	102

Mostly, recoveries were close to 100 %. Only partner 38 reported a value (83%), below 90 %. This results differs significantly (p=0,01; outlier according to Grubbs test) from the recoveries reported by the other participants. Only partner 20 reported a value (108 %), higher than 100 %. In the Grubbs test this result was not regarded as an outlier or straggler.

5.4 Remarks

Table 10: Remarks made by the partners

Partner	Remarks
13	The method is best suitable for nicarbazin determination. We had got no problems.
15	The results on Annex 2 are not corrected for recovery rate
16	During weigh in Nicarbazin standard substance showed hygroscopic attributes
19	It's better to mill the premix. I haven't done that.
20	No remarks
21	The nicarbazin title of the stock solution decreased, after a storage of a month at room temperature, about 15%. This decrease was calculated comparing the two calibration graphs derived from the dilutions, prepared in an interval of 30 days, of the same stock solution. We would like to know the reason of the long time of premixture settlement before dilution in our lab. The settlement was complete after 15 minutes.
23	Not reported
24	No remarks
25	In Nicarbazin method we did some minor modifications in the extraction steps (6.2.2), just to use the same apparatus: - put the flask *in an ultrasonic bath at 50 °C* and sonicate for 15 minutes - mix for 15 minutes using mechanical means - put the flask in an ultrasonic bath *at 50 °C* and sonorate for 15 minutes
26	We experienced no problems with this method.
27	Remarks to the analysis of the premixture: Pouring out the contents of the polyethylene bottle - as it has arrived- on an aluminium foil and mixing the sample on the foil with a spoon and a spatula obviously was not successful: the results of the analyses of four 0.5 g-weight- in portions were: 9684 mg/kg, 10640 mg/kg, 10900 mg/kg, 10216 mg/kg (date of analysis: 19-10-2000) Succeeding mixing of the premixture in the bottle you have sent us - according to your schedule- gave nearly identical results: 11698 mg/kg and 11458 mg/kg (date of analysis: 20-10-2000) Thus I think there should be made a special note in the official text of the method, how to handle not milled premixture samples before analysis.
29	In feed samples it was not necessary to transfer the sample extract quantitatively to a 100 ml volumetric flask (6.2.2). Instead, we used a folded filter to filter the whole extract, and then proceeded the microfiltration step. As for the premixture, we followed the protocol.

Partner	Remarks
30	Test sample size is too small (2,5 g) An extraction of 40 g feed is used with the new method of Mark R. Coleman and all (JAOAC 83, 5, 2000, p. 1027-1038)
31	If the premixture contains 3% nicarbazin, problems may occur with the solubility of nicarbazin and consequently a lower weight or a larger volume of extraction solvent should be applied.
32	No remarks
33	Method has been followed, with the exception of the column and the mobile phase.
35	No remarks
38	Two calibration ranges have been used for quantification of samples. First (0,25 - 5 ppm; n = 8) for feed samples and second (10 - 50 ppm; n = 5) for premixture.
39	No remarks

6 EVALUATION AND CONCLUSIONS

The results of the collaborative study were evaluated in a meeting in Tervuren (Belgium) on June 19-20, 2001.
The task leader for task 1 of the project (Henk Keukens, RIKILT) commented that, applying DMF for preparation of the stock standard solution, negative peaks might emerge in the chromatograms of the calibration solutions that may interfere with the nicarbazin peak. Lab 20 will be asked to prove that no interfering peaks are present by showing a chromatogram of a "blank calibration solution". If this cannot be proved, the results of lab 20 will be discarded.
After the meeting, lab 20 has provided this chromatogram that shows that no interfering peaks are present. For this reason the results of lab 20 are accepted.
Table 7, 20 mg/kg sample: a mistake must be made, lab 24 cannot be a Grubbs double upper straggler while the results of lab 20 are higher.
After the meeting this has been checked: indeed the results of lab 24 are not a Grubbs double upper straggler. Instead, the results of lab 20 are a Grubbs double upper straggler. This has been corrected in Table 7.

The panel has accepted the results of the statistical evaluation, as described in par. 5.1, Table 7. Consequently it can be concluded that for feedingstuffs the repeatability and reproducibility of the method is acceptable. The results obtained for the recovery and for the blind blank samples are also satisfactory. The overall conclusion is that for feedingstuffs the performance of the method is satisfactory.
For the premixture the rsd_R (11,7 %) of the method is too high. According to the panel, a value of approx. 7 % should be attainable. It was decided that a new small-scale collaborative study will be organised (ca. 10 laboratories) with a modified method. The modifications in the method are as follows:

- weight is increased to 1 g
- extraction volume is increased
- more strict description of the calibration method, stating that the concentration of the premixture extract should be in the middle of the calibration curve
- the calibration curve will be shifted to higher concentrations, thus avoiding excessive dilution

The following remarks, related to the method description have been accepted:

- lab 27, mixing of premixture: The handling of the premixture (mixing) prior to the subsampling will be described in the method.
- Lab 31: for premixtures, 2,5 % will be the maximum content (Scope).

ACKNOWLEDGEMENTS

Financial support from the European Commission, DG Research, Standards, Measurements and Testing Programme (SMT) is gratefully acknowledged.
Eli Lilly and Company, Mr. D. Towell, is thanked for supplying the nicarbazin reference standard.
Dr. H. van de Voet, Biometris, Wageningen University and Research Centre is thanked for statistical advice.

APPENDIX 1

Letter with instructions, sent with the samples (with four annexes)

WAGENINGEN UR

RIKILT

DATE
2 October 2000

SUBJECT
collaborative study CANFA
nicarbazin

ENCLOSURE(S)
4

OUR REFERENCE
00/0022090

HANDLED BY
Dr. J. de Jong

DIRECT (TELEPHONE) LINE
+31 317 47 55 81

E-MAIL
j.dejong@RIKILT.WAG-UR.nl

Dear colleague,

Please find enclosed the samples for the collaborative study for nicarbazin:

- 10 feed samples, with the text "additive: NICARBAZIN" and with a sample code; these samples constitute 4 blind duplicates of feed samples containing nicarbazin (contents in the range between 10 and 300 mg/kg) and 1 blind duplicate of a blank feed
- 1 premixture containing nicarbazin, content in the range between 0,5 and 3 %.

For the feed samples you are asked to do a single determination per sample, the premixture must be analysed in duplicate.

For recovery purposes we have included a blank sample, with the text "blank feed for nicarbazin recovery purposes".

The method which has to be used is included as Annex 1 (please note that this method is a *modified* version of the method which was distributed prior to the kick-off meeting).

Annex 2 contains the reporting form. This form will also be send to you by E-mail as an Excel 5.0 file. We strongly prefer to get the results back in electronic form by E-mail (please send the results to the following E-mail address: j.j.m.driessen@rikilt.wag-ur.nl). Of course you can also fill in the form and send it by fax or normal mail.
The **deadline** for reporting the results is **8 December 2000**.

Annex 3 contains instructions for handling (milling, storage) of the samples.

Annex 4 is a questionnaire. We kindly ask you to give us information about the experimental conditions, recoveries, etc.. On this form you can also give your remarks about the method.

The reference standard of nicarbazin which has to be used was already sent to you by mr. Towell (Eli Lilly) in May 2000, Lot Number X47623. In the calculations this reference standard can be regarded as 100 % pure.

RIKILT
State Institute for Quality Control of Agricultural Products
P.O.Box 230
6700 AB Wageningen
The Netherlands

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Building no. 123
Bornsesteeg 45
6708 PD Wageningen

TELEPHONE
+31 317 47 54 00

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+31 317 41 77 17

CHAMBER OF COMMERCE REGISTRATION NO
09098104 te Arnhem

THE INTERNET
www.rikilt.wageningen-ur.nl

QUALIFIED BY STERLAB

We wish you and your colleagues the best with the collaborative studies. If you have any questions, do not hesitate to contact us.

Kind regards,

dr. Jacob de Jong
CANFAS co-ordinator

ing. J.J.M. Driessen
co-ordinator CANFAS
collaborative studies

RIKILT
State Institute for Quality Contr
of Agricultural Products

DATE
2 October 2000

OUR REFERENCE
00/0022090

cc
mrs. I. de Froidmont-Görtz, European Commission, DG Research, CII/3, Brussels
mr. D. Towell, Eli Lilly and Company Ltd., Speke Operations, Liverpool

RIKILT

Annex 1

CANFAS/NIC/18042000/H.KEUKENS

Animal feeding stuffs and premixtures - Determination of NICARBAZIN - High-performance liquid chromatographic method.

1 SCOPE

This operating procedure specifies a method for the determination of the nicarbazin content in animal feeding stuffs and premixtures using high performance liquid chromatography. The limit of quantitation (LOD) determined in the pre-validation study was 20 mg/kg.

2 PRINCIPLE

Samples are extracted by heating in a waterbath, mechanical shaking and sonoration using an acetonitrile/methanol mixture. For feeding stuffs, also water is added. The mixture is transferred in a volumetric flask. After settlement of the solids, an aliquot is filtered and assayed using a reverse-phase isocratic method which measures the 4,4'dinitrocarbanilide (DNC) moiety at a wavelength of 350 nm.

3 REAGENTS

Use only reagents of recognised analytical grade. Use water complying with at least grade 3 in accordance with ISO 3696.

3.1 Acetonitrile, HPLC grade

3.2 Methanol, HPLC grade

3.3 Extraction solvent. Mix 500 ml of acetonitrile (3.1) with 500 ml of methanol (3.2). Mix well using a magnetic stir plate and stir bar.

3.4 Eluent for liquid chromatography. Mix 650 ml acetonitrile (3.1) with 350 ml of purified water. Mix well using a magnetic stir plate and stir bar and degas (e.g. with helium) before use.

3.5 Nicarbazin reference standard.

4 APPARATUS

Jsing laboratory apparatus and, in particular, the following:

1.1 High performance liquid chromatography system consisting of the following:

4.1.1 An autosampler or manual injector set to inject a volume of 20 µl.

4.1.2 A pump set to deliver a constant eluent flow rate of 1,0 ml/min

4.1.3 A column, length 300 mm, internal diameter 3.9 mm, packed with a stationary phase consisting of C-18 material. The particle size should not be smaller than 5 µm and not greater than 10 µm. (A Nova-Pak

column is recommended, but also other columns can be used providing that a satisfactory separation of DNC is achieved).

4.1.4 A detector allowing the measurement of absorbance of UV light at a wavelength of 350 nm, with integrator/recorder.

4.2 Mechanical shaker (e.g. Gyratory shaker, wrist action shaker)

4.3 Micro filters for sample filtration, 0.2 - 0.5 µm

4.4 Mill to prepare laboratory samples with a maximum particle size of 1 mm

4.5 Ultrasonic bath

4.6 Waterbath, 50 °C

4.7 Disposable centrifuge tubes of 50 ml with a screw cap

5 PREPARATION OF THE SAMPLES

5.1 Test samples
The milling and mixing of compound feed samples prior to assay is obligatory. Grind feed samples through a mill (4.4) equipped with a 1 mm screen. After milling, mix the entire sample thoroughly. Store the sample at room temperature in subdued light. Premix samples are not milled.

5.2 Spiked feed samples; 100 mg/kg
Transfer 2.5 ml of the stock standard solution (6.4.1.1) in the sample tube or flask. Evaporate to a small volume (less than 0.5 ml) with a gentle stream of nitrogen, add 2.5 g blank feed, mix thoroughly and wait 10 minutes before starting the extraction procedure by adding water for swelling (see 6.2.2).

6 PROCEDURE

6.1 General
Complete each assay within one working day.

6.2 Extraction

6.2.1 Premixtures

Weigh to the nearest 0.001 g, approximately 0.5 g of the test sample into a 50 ml disposable centrifuge tube (4.7) or directly into a wide neck volumetric flask of 100 ml.

Add 40 ml of extraction solvent (3.3), close the tube or flask and mix manually by swirling.

Put the tubes or flasks in a waterbath of 50 °C for 15 minutes with intermediate swirling at 8 minutes.

Mix thoroughly 15 minutes using a mechanical means (4.2).

Put the tubes or flasks in an ultrasonic bath (4.5) and sonorate for 15 minutes.

Transfer the sample extract if necessary quantitatively in a 100 ml volumetric flask with HPLC eluent (3.4), adjust to volume and mix.

Allow sample solids to settle (minimum 30 minutes).

If additional dilutions are required, dilute the samples with HPLC eluent (3.4) to a final nicarbazin concentration which falls within the standard curve levels.

Filter an aliquot of the final dilution through a micro filter (4.3) for analysis by HPLC.

6.2.2 *Animal feeding stuffs*

Weigh to the nearest 0.01 g, approximately 2.5 g (see remark 9.1) of the test sample into a 50 ml disposable centrifuge tube (4.7) or directly into a wide neck volumetric flask of 100 ml.

Add 5 ml of water. Take care that the whole sample is wetted.

Wait at least 10 minutes.

Add 35 ml of extraction solvent (3.3), close the tube or flask and mix manually by swirling.

Put the tubes or flasks in a waterbath of 50 °C (4.6) for 15 minutes with intermediate swirling at 8 minutes.

Mix thoroughly 15 minutes using a mechanical means (4.2).

Put the tubes or flasks in an ultrasonic bath (4.5) and sonorate for 15 minutes.

Transfer the sample extract if necessary quantitatively in a 100 ml volumetric flask with HPLC eluent (3.4), adjust to volume and mix.

If additional dilutions are required, dilute the samples with HPLC eluent (3.4) to a final nicarbazin concentration which falls within the standard curve levels.

Filter an aliquot of the final dilution through a micro filter (4.3) for analysis by HPLC.

6.3 Determination

6.3.1 Inject 20 µl of the sample extract on to the column of the liquid chromatograph (4.1) and measure the area/height of the DNC peak.

6.3.2 Determine the nicarbazin concentration of the extract by reference to the mean of a calibration curve prepared as described in 6.4 and analysed before and after the sample extracts.

6.4 Calibration

6.4.1 Preparation of nicarbazin standard solutions

6.4.1.1 Nicarbazin stock standard solution, 100 µg/ml

Dissolve 10 mg, weighed to the nearest 0.1 mg, of nicarbazin reference standard (3.5) in 100 ml extraction solvent (3.3). To aid with dissolution, sonoration for approximately 5 minutes is recommended. Mix well. This solution is stable for 24 hours when stored in subdued light at ambient or refrigerated storage conditions (see remark 9.2).

6.4.1.2 Nicarbazin working standard solutions for samples containing 50-250 mg/kg nicarbazin
Prepare a range of calibration working standards containing 0, 1, 2, 3, 4 and 5 µg/ml nicarbazin by diluting the stock standard solution (6.4.1.1) with HPLC eluent (3.4). Working standards must be prepared daily.

6.4.1.3 Nicarbazin working standard solutions for samples containing 20-50 mg/kg nicarbazin
Prepare a range of calibration working standards containing 0; 0.25; 0.5; 1; 2 and 2.5 µg/ml nicarbazin by diluting the stock standard solution (6.4.1.1) with HPLC eluent (3.4). Working standards must be prepared daily.

7 EXPERSSION OF RESULTS

Calculate the nicarbazin content of the test sample by the equation:

$$W_E = \frac{100 \times c}{M} \times f$$

Where:

W_E is the numerical value for the nicarbazin content of the test sample in mg/kg

C is the numerical value of the nicarbazin concentration of the sample extract in µg/ml

M is the numerical value of the mass of the test sample, in g

F is the dilution factor introduced to prepare final sample extracts fitting with the standard curve levels

8 RECOVERY

The recovery obtained for compound feeds should be higher than 90 % at spike levels between 20 and 200 mg/kg.

9 REMARKS

9.1 Homogeneity
For relatively inhomogeneous compound feed samples, the weighed sample amount should be increased to 10 gram with simultaneous up-scaling of the volume of extraction solvent used.

9.2 Solubility
The solubility of the nicarbazin reference standard in extraction solvent is critical. The nicarbazin concentrations in the prepared stock solutions must be monitored by use of a cuvet spectrophotometer as follows. Prepare a solution of 10 µg/ml by diluting the prepared stock standard solution (6.4.1.1) with acetonitrile. Record a UV-Vis spectrum between 220 and 450 nm using a mixture of methanol/acetonitrile (5:95 v/v) as a reference solution. The maximum absorbance measured between 340 and 350 nm should be within a margin of +/- 5 % of the default value. The default value should be established in your own

laboratory by preparing a stock standard solution in duplicate and monitoring the UV-Vis spectra as described above. The default value is the mean result of the duplicates.

9.3 Method characteristics

Precision, repeatability and reproducibility data will be included in the final version of the method description that will be prepared after completion of the collaborative study.

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: Task 4 COLLABORATIVE STUDY

Lab-name:

Contact person:

e-mail:

fax:

telephone:

Date of analysis:

Analyte: NICARBAZIN

Sample code	Unit	Result (mg/kg)
314570		
314590		
314595		
314599		
314609		
314613		
314635		
314636		
314661		
314680		

Sample / Unit	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture		

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 3 - Instructions for handling of the samples

1. Storage

Store the samples at room temperature until analysis

2. Milling (see par. 5.1)

- Feed samples: grind the feed samples with a mill equipped with a 1 mm screen
- Premix: premix samples are not milled

3. Mixing of the test samples before weighing (see par. 5.1)

The container should be filled to a maximum of 50 % of the total volume.
Bring the container in a horizontal position and rotate bottom and top of the container in circles moving the container up and down along the virtual centre of the container for 30 seconds.
Put the container in an upright position and wait a few seconds for settlement of the generated dust.

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Laboratory: .....

Contact person: .....

Date(s) of analysis: .....

Dilution factor of the samples:

- Feed samples (specify for which feed samples): .....
  .....
- Premixture: .....

Chromatographic conditions:

- Column:
  - □ As described in the method
  - □ Other: .....
- Mobile phase:
  - □ As described in the method
  - □ Other: .....
- Flow-rate: ..... ml/min
- Injection volume: .....µl
- Retention time of nicarbazin: ..... min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

Recovery results:

- Percentage recovery: ..... %
- Single / duplicate determinations: □ single □ duplicate
- If duplicate, please give both percentages: ..... % and ..... %
- Spiking level: ..... mg/kg

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Remarks /Comments (if necessary, continue on another page) : .....

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Please complete this questionnaire and return it together with representative chromatograms to:

Ing. J.J.M. Driessen
RIKILT
P.O. Box 230
6700 AE Wageningen
The Netherlands
Fax +31-317-417717

Thank you for your cooperation !

APPENDIX 2

Composition of the feed samples

BESTMIX - Afdruk mengopdracht - 07/09/00 - IPC DIER BARNEVELD

COMPOSITION OF THE FEED SAMPLES

2 134.02 Vleeskuiken I
Rikilt laag vetgehalte
Nicarbazine 20 mg/kg

Broiler, low fat

Grondstof	Silo	%	Gewicht kg	Tol. +/-Afw.	Cumul Gew. kg	Charge 10	Charge
Weegschaal DW 1							
460 Tapioca65%zetmeel	(4)	9.50	47.50	1.43	47.50	..✓..	.....
Weegschaal DW 2							
452 Tarwe EEG (voer)	(9)	23.70	118.50	3.56	118.50	..✓..	.....
450 Mais (E.E.G.)	(12)	25.00	125.00	3.75	243.50	..✓..	.....
Bijstort SP4							
80 Sojabonen get.pell	(0)	5.10	25.50	0.77	25.50	..✓..	.....
84 Sojaschroot 49/3.5rc	(0)	30.09	150.45	4.51	175.95	..✓..	.....
472 Vismeel 72%re deens	(0)	0.40	2.00	0.06	177.95	..✓..	.....
Bijstort SP7							
481 Krijt	(0)	0.60	3.00	0.03	3.00	..✓..	.....
483 Monocalciumfosfaat	(0)	1.26	6.30	0.06	9.30	..✓..	.....
485 Zout	(0)	0.07	0.35	0.00	9.65	..✓..	.....
487 Methionine 100%	(0)	0.20	1.00	0.01	10.65	..✓..	.....
510 Prem kuiken Rikilt	(0)	1.00	5.00	0.05	15.65	..✓..	.....
└ 0,20 % nicarbazin							
Vloeistoffen							
475 Sojaolie	(1)	1.50	7.50	0.23	7.50	..✓..	.....
473 Vet destr.<0.5%polym	(2)	1.58	7.90	0.24	15.40	..✓..	.....
			Totaal :		500.00		

RETOURPRODUKT ....

INSTELLINGEN

T.R. : aut..50%
V.Z. : grof/(fijn) ..80.. %
Z.F. : .2.5.... mm
H.M. : (hoog)/laag toeren
kringloop : ja/(nee)
L.M. : voormengen .60. sec
namengen 300 sec
M.D. : ..=.. l/h

Meel temp : .65.. °C korreltemp. 78°C
Matrijs diam. : 2,5 x 35. mm
K.P. : 23. Amp
Laagdikte Ko : .35 cm
Zeef Ko : .fijn mm
Kruimelen : ja/(neen)
Holmen : 92.0 %
Vocht : %

BESTMIX - Afdruk mengopdracht - 07/09/00 - IPC DIER BARNEVELD

2 134.01 Vleeskuiken I
Rikilt hoog vetgehalte
Nicarbazine 45 mg/kg.

Broiler, high fat

Grondstof	Silo	%	Gewicht kg	Tol. +/-Afw.	Cumul Gew. kg	Charge 9	Charge
Weegschaal DW 1							
464 Raapschroot 34 re	(3)	0.30	1.50	0.05	1.50	V	.....
460 Tapioca65%zetmeel	(4)	15.30	76.50	2.30	78.00	V	.....
Weegschaal DW 2							
452 Tarwe EEG (voer)	(9)	24.94	124.70	3.74	124.70	V	.....
450 Mais (E.E.G.)	(12)	10.00	50.00	1.50	174.70	V	.....
Bijstort SP4							
80 Sojabonen get.pell	(0)	20.00	100.00	3.00	100.00	V	.....
84 Sojaschroot 49/3.5rc	(0)	20.30	101.50	3.05	201.50	V	.....
Bijstort SP7							
481 Krijt	(0)	0.57	2.85	0.03	2.85	V	.....
483 Monocalciumfosfaat	(0)	1.27	6.35	0.06	9.20	V	.....
485 Zout	(0)	0.08	0.40	0.00	9.60	V	.....
486 L-lysine HCl	(0)	0.01	0.05	0.00	9.65	V	.....
487 Methionine 100%	(0)	0.23	1.15	0.01	10.80	V	.....
510 Prem kuiken Rikilt L 0,45 % nicarbazin	(0)	1.00	5.00	0.05	15.80	V	.....
Vloeistoffen							
473 Vet destr.<0.5%polym	(2)	6.00	30.00	0.90	30.00	V	.....
			Totaal :		500.00		

RETOURPRODUKT ....

INSTELLINGEN

T.R. : aut. 50%
V.Z. : grof/(fijn) 80 %
Z.F. : 2,5 mm
H.M. : (hoog)/laag toeren
kringloop : ja/(nee)
L.M. : voormengen 60 sec
namengen 300 sec
M.D. : 1/h

Meel temp : 59°C korreltemp. 68 °C
Matrijs diam. : 2,5 x 35 mm
K.P. : 23 Amp
Laagdikte Ko : 35 cm
Zeef Ko : fijn mm
Kruimelen : ja/(neen)
Holmen : 76,6 %
Vocht : %

BESTMIX - Afdruk mengopdracht - 08/09/00 - IPC DIER BARNEVELD

2 134.01 Vleeskuiken I
Rikilt hoog vetgehalte
Nicarbazine 110 mg/kg

Broiler, high fat

Grondstof	Silo	%	Gewicht kg	Tol. +/-Afw.	Cumul Gew. kg	Charge //	Charge
Weegschaal DW 1							
464 Raapschroot 34 re	(3)	0.30	1.50	0.05	1.50	✓	.....
460 Tapioca65%zetmeel	(4)	15.30	76.50	2.30	78.00	✓	.....
Weegschaal DW 2							
452 Tarwe EEG (voer)	(9)	24.94	124.70	3.74	124.70	✓	.....
450 Mais (E.E.G.)	(12)	10.00	50.00	1.50	174.70	✓	.....
Bijstort SP4							
80 Sojabonen get.pell	(0)	20.00	100.00	3.00	100.00	✓	.....
84 Sojaschroot 49/3.5rc	(0)	20.30	101.50	3.05	201.50	✓	.....
Bijstort SP7							
481 Krijt	(0)	0.57	2.85	0.03	2.85	✓	.....
483 Monocalciumfosfaat	(0)	1.27	6.35	0.06	9.20	✓	.....
485 Zout	(0)	0.08	0.40	0.00	9.60	✓	.....
486 L-lysine HCl	(0)	0.01	0.05	0.00	9.65	✓	.....
487 Methionine 100%	(0)	0.23	1.15	0.01	10.80	✓	.....
510 Prem kuiken Rikilt L 1,1 % nicarbazin	(0)	1.00	5.00	0.05	15.80	✓	.....
Vloeistoffen							
473 Vet destr.<0.5%polym	(2)	6.00	30.00	0.90	30.00	✓	.....
			Totaal :		500.00		

RETOURPRODUKT ....

INSTELLINGEN

T.R. : aud. 50%
V.Z. : grof/fijn ..80.. %
Z.F. : 2.5..... mm
H.M. : hoog/laag toeren
kringloop : ja/nee
L.M. : voormengen .60. sec
namengen 300. sec
M.D. : ...... l/h

Meel temp : 64... °C korrel temp 72 °C
Matrijs diam. : 2.5. x 35. mm
K.P. : 22.. Amp
Laagdikte Ko : 35. cm
Zeef Ko : fijn mm
Kruimelen : ja/neen
Holmen : 79 %
Vocht : %

BESTMIX - Afdruk mengopdracht - 08/09/00 - IPC DIER BARNEVELD

2 134.02 Vleeskuiken I
Rikilt laag vetgehalte
Nicarbazine 240 mg/kg

Broiler, low fat

Grondstof	Silo	%	Gewicht kg	Tol. +/-Afw.	Cumul Gew. kg	Charge 12	Charge
Weegschaal DW 1							
460 Tapioca65%zetmeel	(4)	9.50	47.50	1.43	47.50	V	
Weegschaal DW 2							
452 Tarwe EEG (voer)	(9)	23.70	118.50	3.56	118.50	V	
450 Mais (E.E.G.)	(12)	25.00	125.00	3.75	243.50	V	
Bijstort SP4							
80 Sojabonen get.pell	(0)	5.10	25.50	0.77	25.50	V	
84 Sojaschroot 49/3.5rc	(0)	30.09	150.45	4.51	175.95	V	
472 Vismeel 72%re deens	(0)	0.40	2.00	0.06	177.95	V	
Bijstort SP7							
481 Krijt	(0)	0.60	3.00	0.03	3.00	V	
483 Monocalciumfosfaat	(0)	1.26	6.30	0.06	9.30	V	
485 Zout	(0)	0.07	0.35	0.00	9.65	V	
487 Methionine 100%	(0)	0.20	1.00	0.01	10.65	V	
510 Prem kuiken Rikilt	(0)	1.00	5.00	0.05	15.65	V	
↳ 2,4 % nicarbazin							
Vloeistoffen							
475 Sojaolie	(1)	1.50	7.50	0.23	7.50	V	
473 Vet destr.<0.5%polym	(2)	1.58	7.90	0.24	15.40	V	
			Totaal :		500.00		

RETOURPRODUKT ....

INSTELLINGEN

T.R. : aut. 50%
V.Z. : grof/fijn 86 %
Z.F. : 2.5 mm
H.M. : hoog/laag toeren
kringloop : ja/nee
L.M. : voormengen 60 sec
namengen 300 sec
M.D. : 7 l/h

Meel temp : 62 °C koneltemp 77°C
Matrijs diam. : 2.5 x 35 mm
K.P. : 23 Amp
Laagdikte Ko : 35 cm
Zeef Ko : fijn mm
Kruimelen : ja/neen
Holmen : 88,2 %
Vocht : %

APPENDIX 3

Homogeneity of samples

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Homogeneity test collaborative study

Additive : **Nicarbazin**
Product : **Feed sample: 20 ppm**

Date of determination : September 19th, 2000

Sample	Content mg/kg	Duplicate average mg/kg
18454 A	21,7	22,2
18454 B	22,7	
18455 A	27,0	23,4
18455 B	19,8	
18456 A	26,0	25,1
18456 B	24,1	
18457 A	23,5	22,8
18457 B	22,1	
18458 A	22,6	22,1
18458 B	21,5	
18459 A	23,0	24,0
18459 B	24,9	
18460 A	21,2	21,3
18460 B	21,3	
18461 A	22,0	22,6
18461 B	23,2	
18462 A	21,3	20,5
18462 B	19,7	
18463 A	20,4	20,1
18463 B	19,8	

Homogeneity OK Criterion : $CV_{between}$ = < 8%		
Average	**22,4**	
SD (between samples)	**1,53**	
CV (between samples)	**6,8**	Result Grubb's test
Grubb's test, single lower	**1,499**	**no outlier**
Grubb's test, single upper	**1,731**	**no outlier**
Grubb's test, double lower	**0,4746**	**no outliers**
Grubb's test, double upper	**0,4462**	**no outliers**

Repeatability		
SD (within samples)	(sd_r)	**1,86**
CV (within samples)	(CV (%))	**8,3**

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Homogeneity test collaborative study

Additive : Nicarbazin
Product : Feed sample: 45 ppm

Date of determination : September 19th, 2000

Sample	Content mg/kg	Duplicate average mg/kg
18464 A	49,0	48,7
18464 B	48,3	
18465 A	45,4	44,4
18465 B	43,4	
18466 A	49,2	49,9
18466 B	50,5	
18467 A	48,5	49,9
18467 B	51,4	
18468 A	46,6	44,8
18468 B	43,0	
18469 A	49,6	49,3
18469 B	49,0	
18470 A	45,4	46,4
18470 B	47,4	
18471 A	43,1	43,6
18471 B	44,1	
18472 A	45,1	47,3
18472 B	49,4	
18473 A	49,7	50,3
18473 B	50,9	

Homogeneity OK Criterion : $CV_{between}$ = < 8%		
Average	**47,4**	
SD (between samples)	**2,53**	
CV (between samples)	**5,3**	Result Grubb's test
Grubb's test, single lower	**1,522**	**no outlier**
Grubb's test, single upper	**1,124**	**no outlier**
Grubb's test, double lower	**0,4728**	**no outliers**
Grubb's test, double upper	**0,6901**	**no outliers**

Repeatability		
SD (within samples)	(sd_r)	**1,62**
CV (within samples)	(CV (%))	**3,4**

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Homogeneity test collaborative study

Additive : **Nicarbazin**
Product : **Feed sample: 110 ppm**

Date of determination : September 20th, 2000

Sample	Content mg/kg	Duplicate average mg/kg
18474 A	117,9	123,1
18474 B	128,3	
18475 A	108,2	114,1
18475 B	120,0	
18476 A	120,4	113,4
18476 B	106,5	
18477 A	121,1	121,3
18477 B	121,6	
18478 A	128,0	128,8
18478 B	129,7	
18479 A	118,3	120,0
18479 B	121,7	
18480 A	129,1	122,7
18480 B	116,4	
18481 A	121,2	117,3
18481 B	113,4	
18482 A	130,5	132,8
18482 B	135,1	
18483 A	123,4	122,8
18483 B	122,2	

Homogeneity OK Crtiterion : $CV_{between}$ = < 8%		
Average	**121,6**	
SD (between samples)	**6,030**	
CV (between samples)	**5,0**	Result Grubb's test
Grubb's test, single lower	**1,362**	**no outlier**
Grubb's test, single upper	**1,847**	**no outlier**
Grubb's test, double lower	**0,5233**	**no outliers**
Grubb's test, double upper	**0,3343**	**no outliers**

Repeatability		
SD (within samples)	(sd_r)	**5,911**
CV (within samples)	(CV (%))	**4,9**

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Homogeneity test collaborative study

Additive : Nicarbazin
Product : Feed sample: 240 ppm

Date of determination : September 20th, 2000

Sample	Content mg/kg	Duplicate average mg/kg
18484 A	255,0	265,1
18484 B	275,2	
18485 A	250,7	271,9
18485 B	293,1	
18486 A	267,2	261,5
18468 B	255,8	
18487 A	277,6	272,8
18487 B	267,9	
18488 A	261,0	261,4
18488 B	261,9	
18489 A	268,0	266,9
18489 B	265,7	
18490 A	256,3	257,3
18490 B	258,2	
18491 A	266,6	267,3
18491 B	268,0	
18492 A	261,8	260,9
18492 B	259,9	
18493 A	248,2	253,0
18493 B	257,8	

Homogeneity OK Criterion : $CV_{between}$ = < 8%		
Average	**263,8**	
SD (between samples)	**6,22**	
CV (between samples)	**2,4**	Result Grubb's test
Grubb's test, single lower	**1,737**	**no outlier**
Grubb's test, single upper	**1,442**	**no outlier**
Grubb's test, double lower	**0,4336**	**no outliers**
Grubb's test, double upper	**0,4761**	**no outliers**

Repeatability		
SD (within samples)	(sd_r)	**11,28**
CV (within samples)	(CV (%))	**4,3**

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Homogeneity test collaborative study

Additive : **Nicarbazin**
Product : **Premixture: 1.1%**

Date of determination : September 21th, 2000

Sample	Content %	Duplicate average %
16129-1A	1,19	1,19
16129-1B	1,20	
16129-2A	1,22	1,16
16129-2B	1,09	
16129-3A	1,04	1,07
16129-3B	1,11	
16129-4A	1,07	1,12
16129-4B	1,16	
16129-5A	1,11	1,14
16129-5B	1,17	
16129-6A	1,15	1,17
16129-6B	1,19	
16129-7A	1,16	1,13
16129-7B	1,10	
16129-8A	1,10	1,09
16129-8B	1,08	
16129-9A	1,29	1,22
16129-9B	1,15	
16129-10A	1,24	1,21
16129-10B	1,18	

Homogeneity **OK**		
Crtiterion : $CV_{between}$ = < 7%		
Average	**1,15**	
SD (between samples)	**0,049**	
CV (between samples)	**4,3**	Result Grubb's test
Grubb's test, single lower	**1,543**	**no outlier**
Grubb's test, single upper	**1,399**	**no outlier**
Grubb's test, double lower	**0,4532**	**no outliers**
Grubb's test, double upper	**0,5254**	**no outliers**

Repeatability		
SD (within samples)	(sd_r)	**0,055**
CV (within samples)	(CV (%))	**4,8**

APPENDIX 4

Sample codes

Sample codes supplied to the participants in the nicarbazin collaborative study

NICARBAZIN number of participants 19	NIC broiler 20ppm NIC 1a	NIC broiler 20ppm NIC 1b	NIC broiler 45ppm NIC 2a	NIC broiler 45 ppm NIC 2b	NIC broiler 110ppm NIC 3a	NIC broiler 110 ppm NIC 3b	NIC broiler 240 ppm NIC 4a	NIC broiler 240 ppm NIC 4b	VIRG broiler 2ppm NIC blank 1a	VIRG broiler 2ppm NIC blank 1b
Participant code										
13	134653	134600	134666	134692	134641	134632	134642	134559	134657	134535
15	154604	154549	154597	154538	154668	154522	154610	154677	154514	154543
16	164520	164671	164611	164557	164639	164656	164519	164631	164523	164515
19	194647	194616	194676	194585	194601	194545	194627	194655	194508	194553
20	204571	204575	204634	204504	204683	204645	204583	204587	204686	204688
21	214516	214577	214663	214531	214606	214673	214684	214503	214580	214526
23	234552	234525	234524	234607	234582	234598	234547	234652	234596	234510
24	244550	244586	244505	244630	244528	244513	244578	244622	244619	244533
25	254685	254536	254658	254689	254540	254659	254602	254554	254551	254527
26	264603	264529	264664	264537	264544	264679	264555	264648	264518	264566
27	274556	274511	274548	274660	274569	274623	274618	274691	274558	274539
29	294644	294530	294646	294628	294624	294629	294612	294650	294614	294594
30	304532	304563	304626	304568	304584	304521	304682	304562	304589	304667
31	314635	314590	314613	314595	314570	314680	314599	314636	314661	314609
32	324640	324593	324561	324517	324507	324625	324576	324506	324591	324675
33	334608	334565	334605	334670	334567	334573	334615	334541	334572	334638
35	354621	354546	354581	354534	354678	354662	354687	354574	354690	354651
38	384509	384592	384649	384674	384564	384512	384579	384542	384633	384560
39	394588	394665	394681	394669	394617	394643	394620	394637	394654	394672

APPENDIX 5

Nicarbazin reference standard profile

Tel: (0)151-448-6406
Fax: (0)151-448-6060

ELI LILLY COMPANY LIMITED - SPEKE OPERATIONS

Fleming Road
Speke, Liverpool L24 9LN UK

May 22nd 2000

CANFAS STUDY - NICARBAZIN REFERENCE STANDARD

The following has been extracted from the Lilly Nicarbazin reference standard profile:

REFERENCE STANDARD PROFILE

*	Effective Date:	October 29, 1998	Compound:	093760
*	Supersedes:	October 11, 1995	Revision:	10
*	Expiry Date:	October 28, 2001	Standard:	1994

Name: Nicarbazin
Chemical Name: 4,4'-Dinitrocarbanilide 4,6-dimethyl-2-pyrimidinol complex
Lot Number: X47623
* Defined Potency: 69.6% 4,4'-dinitrocarbanilide ($C_{13}H_{10}N_4O_5$) or 27.0% 4,6-dimethyl-2-pyrimidinol ($C_6H_8N_2O$).

Handling: Normal laboratory precautions should be used.
Storage: Tightly closed amber glass bottle at room temperature, 15° to 30°C.

D. P. Towell

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 13

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: Task 4 COLLABORATIVE STUDY
Lab-name:
Contact person: e-mail:
fax:
telephone:
Date of analysis:

Analyte: NICARBAZIN

Sample code	Unit	Result (mg/kg)
134535		0
134559		275
134600		23
134632		128
134641		124
134642		270
134653		21
134657		0
134666		46
134692		46

Sample / Unit	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture	10292	10933

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Date(s) of analysis: .....

Dilution factor of the samples:

- Feed samples (specify for which feed samples): ..... 2,5g / 100 = µg/ml * 100 * 1/25 = mg/kg .....
- Premixture: ..... 0,5g / 100/2,5 / 50 = µg/ml * 50 * 100/25 * 1/0,5 = mg/kg

Chromatographic conditions:

- Column:
  - ☒ As described in the method
  - ☐ Other: .....
- Mobile phase:
  - ☒ As described in the method
  - ☐ Other: .....
- Flow-rate: ..1,0..... ml/min
- Injection volume: ..20.....µl
- Retention time of nicarbazin: ..4,9.... min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

Recovery results:

- Percentage recovery: ..98.... %
- Single / duplicate determinations: ☐ single ☒ duplicate
- If duplicate, please give both percentages: ..97.. % and ..99.. %
- Spiking level: ..250.... mg/kg

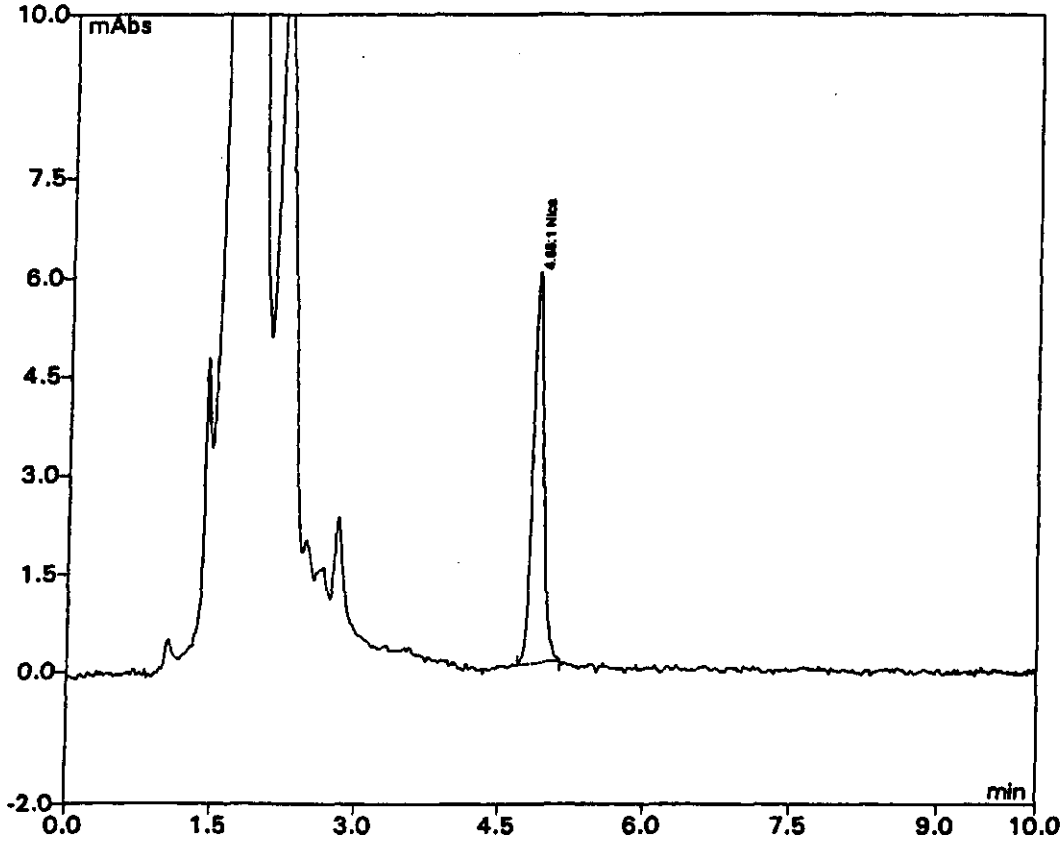
KromaSystem 2000

Channel 3

KromaSystem 2000 Version 1.83 RESULT REPORT: INTEGRATION

SYS1 - NICAR006.SMP (modified): Nicarbazin-Enquete
No. 18: F1813'' 2.5g/100ml Acquired : 11.12.00 16:23:00
Channel 3: DAD 3 chrom. 340/ 5 Processed: 14.12.00 10:53
No Text

Program File ..... NICAR1 ..... Nicarbazin-Methode
Worksheet ..... NICAR1 ..... Nicarbacin-Best
Peak Table ..... NICAR .....
Parameter Table .. NICAR .....
Report File ....
Document File ....



No.	PNo	Ret.Time min	Type	Name	Area mAbs*min	Amount	Rel.Ar %
1	1	4.68	MOD	Nicarbacin	7.9585e-001	2.2549e+001	100.00
					7.9585e-001	2.2549e+001	100.00

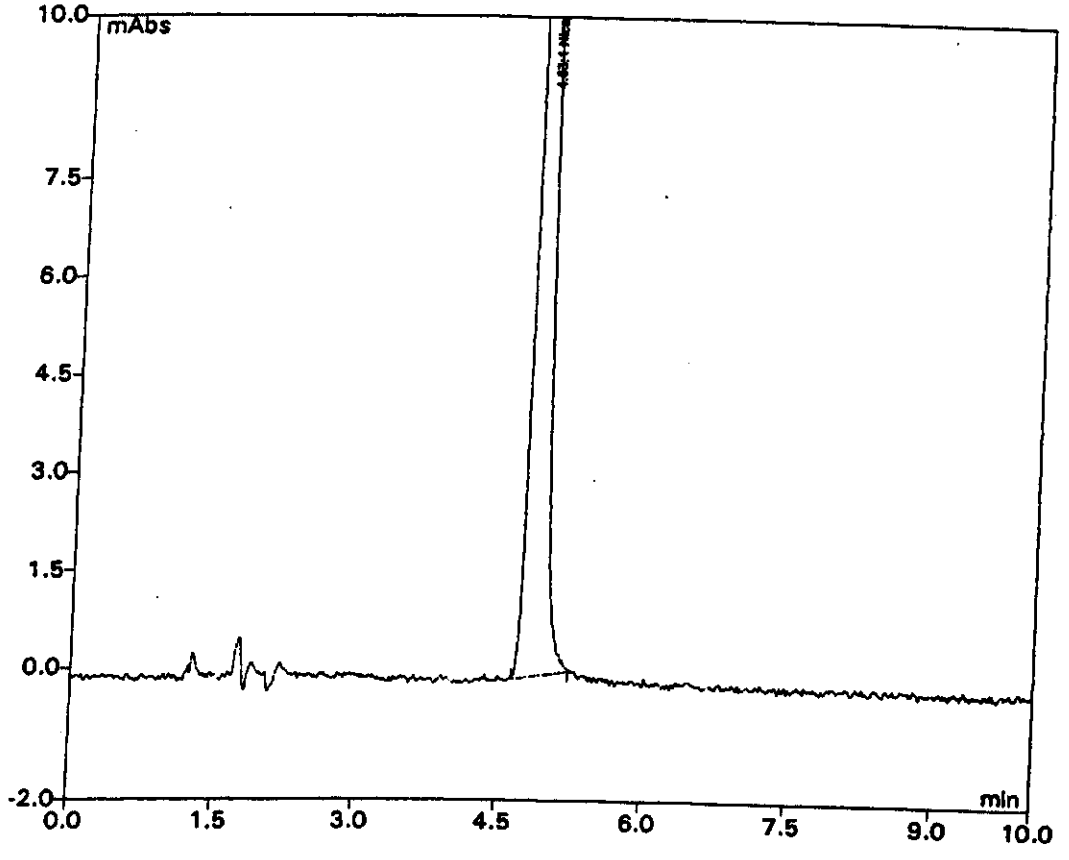
mg/kg

Channel 3

KromaSystem 2000 Version 1.83 RESULT REPORT: INTEGRATION

SYS1 - NICAR007.SMP (modified): Nicarbacin-Enquete
No. 29: F1822'0.5g/100ml/2.5ml/50ml Acquired : 14.12.00 12:53:05
Channel 3: DAD 3 chrom. 340/ 5 Processed: 14.12.00 13:32
No Text

Program File ..... NICAR1 ..... Nicarbazin-Methode
Worksheet ..... NICAR1 ..... Nicarbacin-Best
Peak Table ..... NICAR .....
Parameter Table .. NICAR .....
Report File ....
Document File ....



No.	PNo	Ret.Time min	Type	Name	Area mAbs*min	Amount	Rel.Ar %
1	1	4.83	MLR	Nicarbacin	3.5444e+000	1.0292e+004	100.00
					3.5444e+000	1.0292e+004	100.00

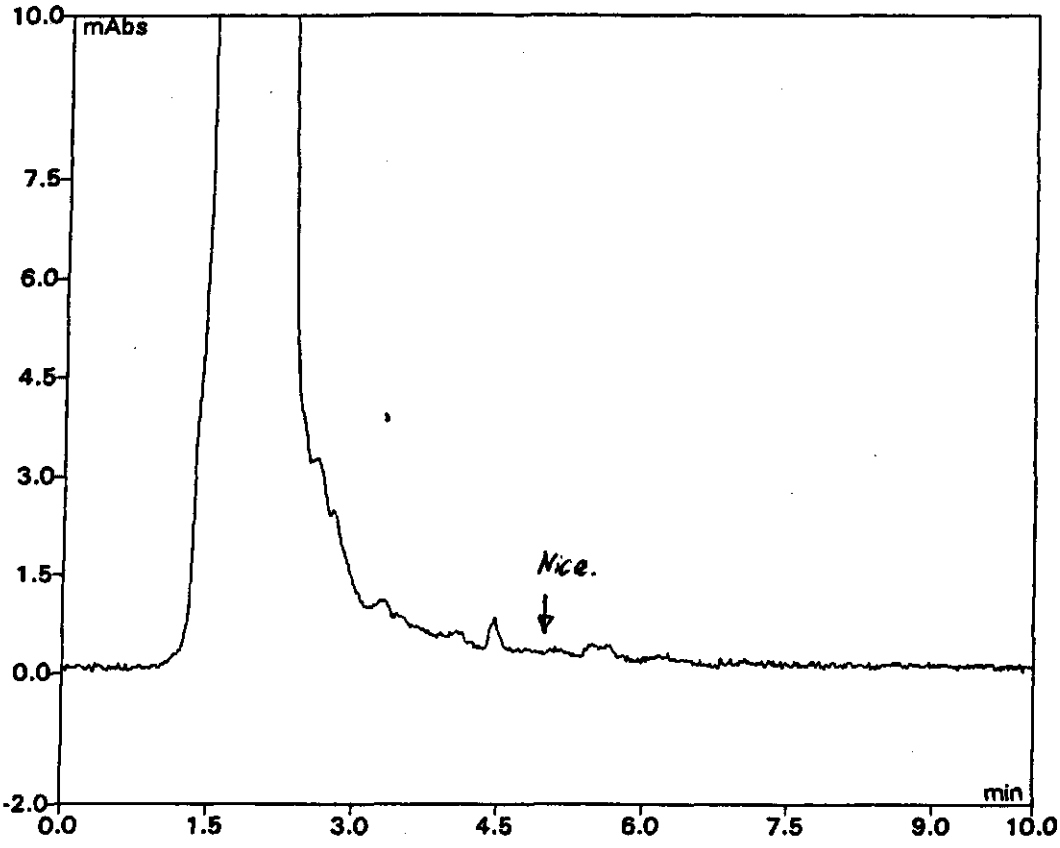
mg/kg

Channel 3

KromaSystem 2000 Version 1.83 RESULT REPORT: INTEGRATION

SYS1 - NICAR006.SMP (modified): Nicarbazin-Enquete
No. 11: F1811' 2.5g/100ml Acquired : 11.12.00 15:07:01
Channel 3: DAD 3 chrom. 340/ 5 Processed: 14.12.00 10:52
No Text

Program File ..... NICAR1 ..... Nicarbazin-Methode
Worksheet ..... NICAR1 ..... Nicarbacin-Best
Peak Table ..... NICAR .....
Parameter Table .. NICAR .....
Report File ....
Document File ....



No.	PNo	Ret.Time min	Type	Name	Area mAbs*min	Amount	Rel.Ar %
					0.0000e+000	0.0000e+000	0.00

mg/kg

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 15

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: **Task 4 COLLABORATIVE STUDY**
Lab-name:
Contact person: e-mail:
fax:
telephone:
Date of analysis:

Analyte: **NICARBAZIN**

	Unit	Result (mg/kg)
Sample code		
154514		blank
154522		117,7
154538		44,8
154543		blank
154549		27,9
154597		48,3
154604		22,5
154610		269
154668		124,2
154677		287,3

Unit / Sample	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture	10640	10970

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Date(s) of analysis: 25 OCTOBER 2000

Dilution factor of the samples:

- Feed samples (specify for which feed samples): DILUTION = 2 FOR SAMPLES 154610 AND 154677
- Premixture: DILUTION = 25

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ☒ Other: HYPERSIL ODS 5µm 200X4.6 mm + GUARD COLUMN
- Mobile phase:
  - ☐ As described in the method
  - ☒ Other: WATER 50% / ACETONITRILE 50%
- Flow-rate: 1.0 ml/min
- Injection volume: 20 µl
- Retention time of nicarbazin: 6.8 min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

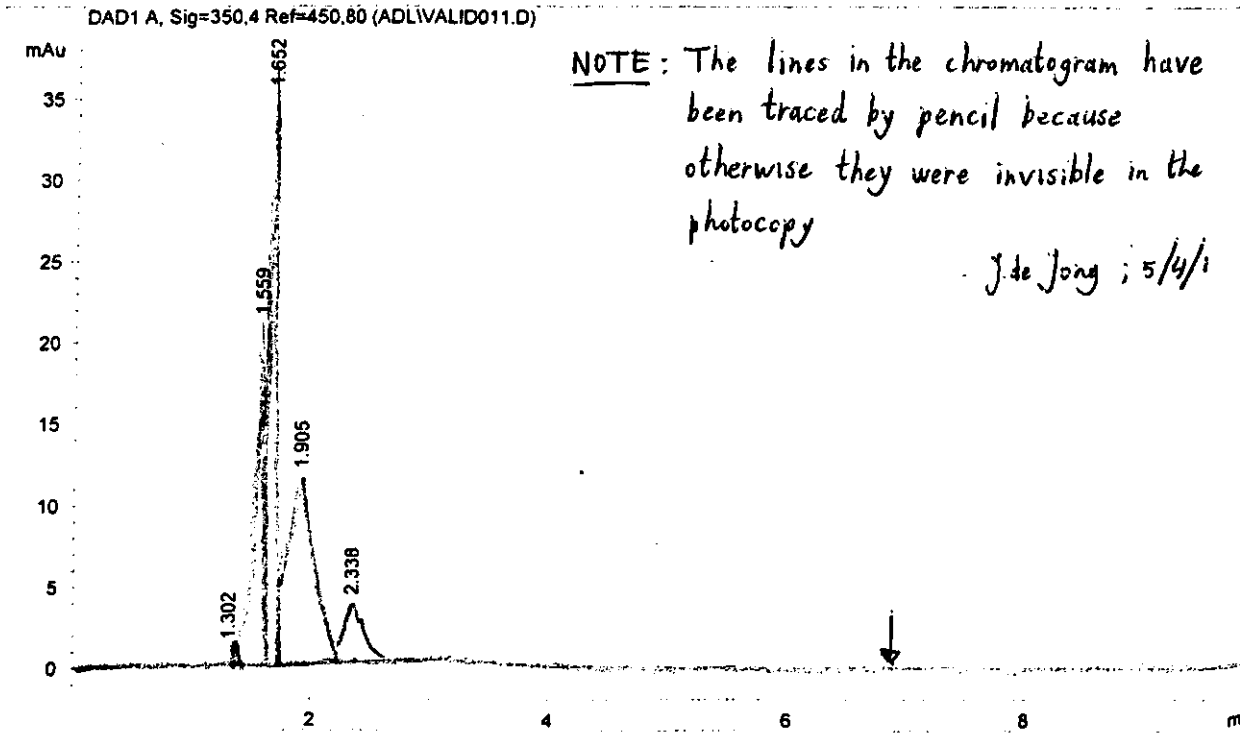
Recovery results:

- Percentage recovery: 93.1%
- Single / duplicate determinations: ☐ single ☐ duplicate
- If duplicate, please give both percentages: 89.0 % and 95.2 %
- Spiking level: 100 mg/kg

Data File C:\HPCHEM\1\DATA\ADL\VALID011.D Sample Name: 5

154 514

Injection Date	: 24/10/00 17.10.00	Seq. Line	: 12
Sample Name	: 514	Vial	: 11
Acq. Operator	: adl	Inj	: 1
		Inj Volume	: 20 µl
Acq. Method	: C:\HPCHEM\1\METHODS\MNICVALI.M		
Last changed	: 24/10/00 15.05.48 by adl		
Analysis Method	: C:\HPCHEM\1\METHODS\MNICVALI.M		
Last changed	: 25/10/00 7.55.17 by adl (modified after loading)		



NOTE: The lines in the chromatogram have been traced by pencil because otherwise they were invisible in the photocopy

J. de Jong ; 5/4/1

External Standard Report

Sorted By	:	Signal
Calib. Data Modified	:	Wednesday 25 October ~~1900~~ 7.43.37
Multiplier	:	1.0000
Dilution	:	40.0000
Uncalibrated Peaks	:	not reported

Signal 1: DAD1 A, Sig=350,4 Ref=450,80
Results obtained with enhanced integrator!

RetTime [min]	Type	Area [mAu*s]	Amt/Area	Amount [ng/ul]	Grp	Name
6.874		-	-	-		Nicarbazin

Totals : 0.00000

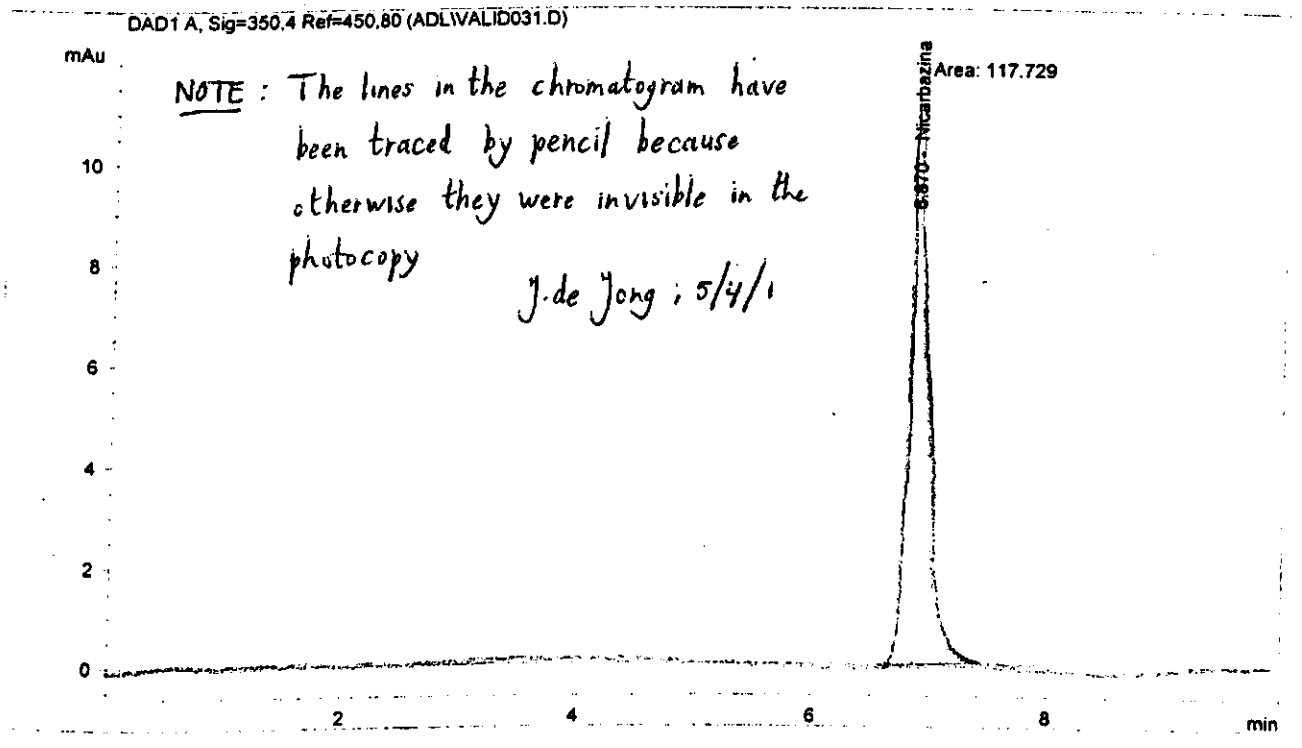
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Data File C:\HPCHEM\1\DATA\ADL\VALID031.D Sample Name: pre. m.

PREMIXTURE

Injection Date	: 24/10/00 20.53.01	Seq. Line :	32
Sample Name	: pre. m.	Vial :	22
Acq. Operator	: adl	Inj :	1
		Inj Volume :	20 µl
Acq. Method	: C:\HPCHEM\1\METHODS\MNICVALI.M		
Last changed	: 24/10/00 15.05.48 by adl		
Analysis Method	: C:\HPCHEM\1\METHODS\MNICVAL1.M		
Last changed	: 25/10/00 9.54.34 by adl (modified after loading)		



NOTE : The lines in the chromatogram have been traced by pencil because otherwise they were invisible in the photocopy

J. de Jong ; 5/4/1

External Standard Report

Sorted By	:	Signal
Calib. Data Modified	:	25/10/00 9.54.29
Multiplier	:	1.0000
Dilution	:	5.000e3
Uncalibrated Peaks	:	not reported

Signal 1: DAD1 A, Sig=350,4 Ref=450,80
Results obtained with enhanced integrator!

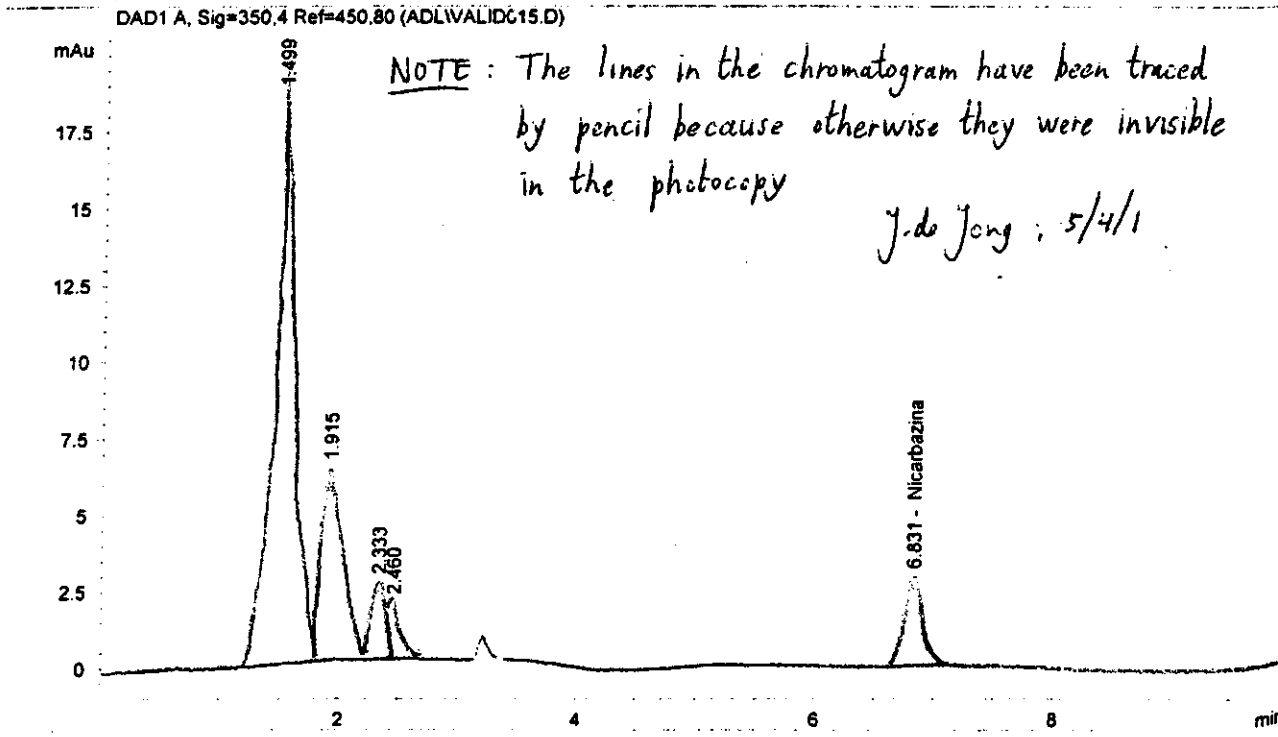
RetTime [min]	Type	Area [mAu*s]	Amt/Area	Amount [ng/ul]	Grp	Name
6.870	MM	117.72948	1.80819e-2	1.06439e4		Nicarbazin
Totals :				1.06439e4		

*** End of Report ***

Data File C:\HPCHEM\1\DATA\ADL\VALID015.D Sample Name: 60

154604

Injection Date	: 24/10/00 17.54.38	Seq. Line	: 16
Sample Name	: 604	Vial	: 15
Acq. Operator	: adl	Inj	: 1
		Inj Volume	: 20 µl
Acq. Method	: C:\HPCHEM\1\METHODS\MNICVALI.M		
Last changed	: 24/10/00 15.05.48 by adl		
Analysis Method	: C:\HPCHEM\1\METHODS\MNICVALI.M		
Last changed	: 25/10/00 7.55.17 by adl (modified after loading)		



NOTE: The lines in the chromatogram have been traced by pencil because otherwise they were invisible in the photocopy

J. de Jong ; 5/4/1

External Standard Report

Sorted By	:	Signal
Calib. Data Modified	:	Wednesday 25 October ~~1900~~ 7.43.37
Multiplier	:	1.0000
Dilution	:	40.0000
Uncalibrated Peaks	:	not reported

Signal 1: DAD1 A, Sig=350,4 Ref=450,80
Results obtained with enhanced integrator!

RetTime [min]	Type	Area [mAu*s]	Amt/Area	Amount [ng/ul]	Grp	Name
6.831	BB	30.38545	1.85038e-2	22.48985		Nicarbazin
Totals :				22.48985		

*** End of Report ***

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 16

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: **Task 4 COLLABORATIVE STUDY**
Lab-name:
Contact person: e-mail:
fax:
telephone:
Date of analysis:

Analyte: **NICARBAZIN**

Sample code	Unit	Result (mg/kg)
164515		not found
164519		244,8
164520		17,54
164523		not found
164557		44,88
164611		45,85
164631		245,36
164639		112,78
164656		104,84
164671		19,22

Sample	Unit	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture		10345	10018

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Date(s) of analysis: 2000-11-21, 2000-11-22, 2000-11-23 (each assay one day)

Dilution factor of the samples:

- Feed samples (specify for which feed samples): —
- Premixture: 25

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - x Other: Spherisorb ODS 1, 5 µm, 250 x 4 mm
- Mobile phase:
  - x As described in the method
  - ☐ Other:
- Flow-rate: 1.0 ml/min
- Injection volume: 20 µl
- Retention time of nicarbazin: 5.3 min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

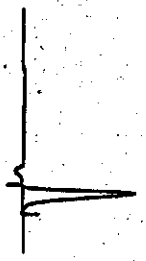
Please indicate the nicarbazin peak with an arrow

Recovery results:

- Percentage recovery: 101.6 %
- Single / duplicate determinations: ☐ single x duplicate
- If duplicate, please give both percentages: 101.1 and 102.1 %
- Spiking level: 104 mg/kg

Nicarbazin

CH. 1 C.S 5.00 ATT 5 OFFS 10 11/23/00 10:36



Standard 1060 µg/ml

16

INJ NO. OF STD : 1 / 1 REP , 1st level

D-2500 11/23/00 10:36

METHOD: ALLGEMEIN TAG: 608 CH: 1

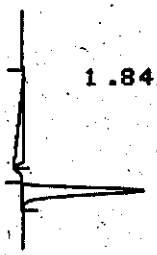
FILE: 3 CALC-METHOD: EXT-STD TABLE: 9 CONC: HEIGHT

NO.	RT	AREA	HEIGHT	UG/ML	NAME
1	5.31	45947	3172	1.060	NICARB

CH. 1 C.S 5.00 ATT 5 OFFS 10 11/23/00 10:44

Remix

10.345 mg/kg



D-2500 11/23/00 10:44

METHOD: ALLGEMEIN TAG: 609 CH: 1

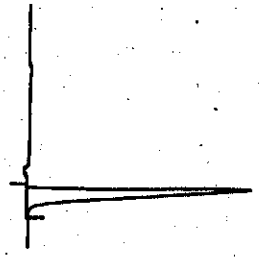
FILE: 3 CALC-METHOD: EXT-STD TABLE: 9 CONC: HEIGHT

NO.	RT	AREA	HEIGHT	UG/ML	NAME
1	1.84	20319	44	0.000	
2	5.31	49912	3450	1.153	NICARB
TOTAL		70231	3494	1.153	

PEAK REJ : 10
SF : 1.000
SAMP-AMT : 1.000

CH. 1 C.S 5.00 ATT 5 OFFS 10 11/24/00 10:34

Nicarbazin
Standard 2,140 µg/ml



16

INJ NO. OF STD : 1 / 1 REP , 1st level

D-2500 11/24/00 10:34

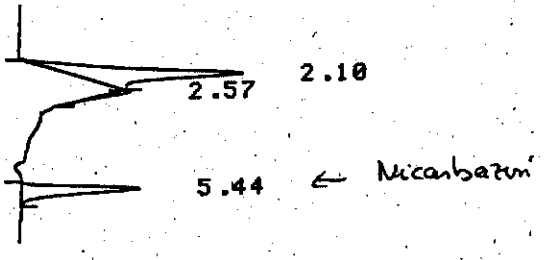
METHOD: ALLGEMEIN TAG: 639 CH: 1

FILE: 3 CALC-METHOD: EXT-STD TABLE: 9 CONC: HEIGHT

NO.	RT	AREA	HEIGHT	UG/ML	NAME
1	5.47	95187	6404	2.140	NICARB

CH. 1 C.S 5.00 ATT 5 OFFS 10 11/24/00 10:44

Sample 164557
44,9 mg/kg



D-2500 11/24/00 10:44

METHOD: ALLGEMEIN TAG: 640 CH: 1

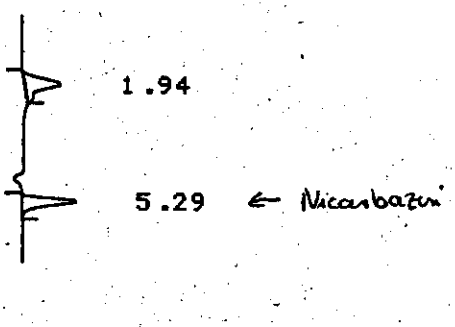
FILE: 3 CALC-METHOD: EXT-STD TABLE: 9 CONC: HEIGHT

NO.	RT	AREA	HEIGHT	UG/ML	NAME
1	2.10	96693	4672	0.005	
2	2.57	6819	467	0.000	
3	5.44	48721	3348	1.119	NICARB
TOTAL		152233	8487	1.124	

PEAK REJ : 50
SF : 1.000
SAMP-AMT : 1.000

Nicarbazin

CH. 1 C.S 5.00 ATT 5 OFFS 10 11/23/00 12:50



standard 0,53 µg/ml

16

INJ NO. OF STD : 1 / 1 REP , 1st level

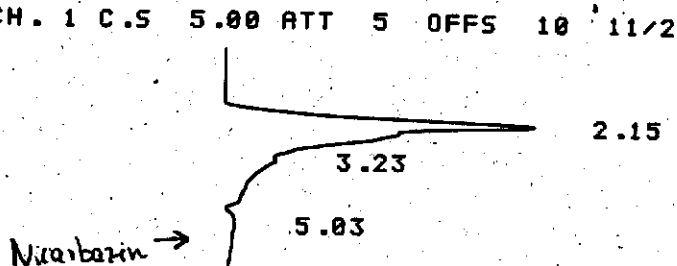
D-2500 11/23/00 12:50

METHOD: ALLGEMEIN TAG: 622 CH: 1

FILE: 3 CALC-METHOD: EXT-STD TABLE: 9 CONC: HEIGHT

NO.	RT	AREA	HEIGHT	UG/ML	NAME
2	5.29	22429	1561	0.530	NICARB

CH. 1 C.S 5.00 ATT 5 OFFS 10 11/23/00 13:09



Sample 164523

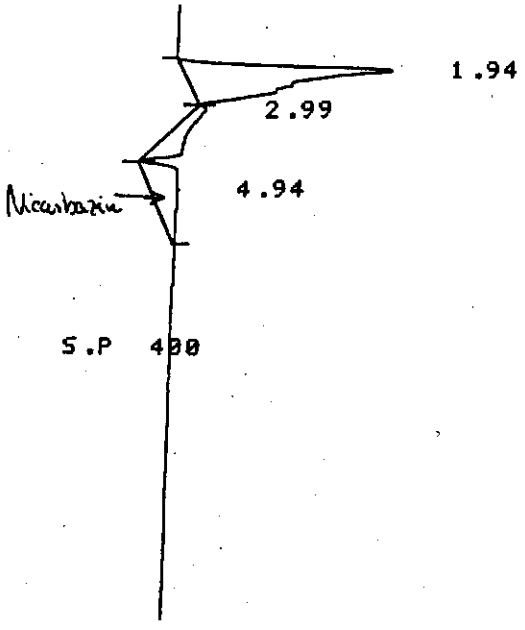
ESCAPE

Nicarbazin

16

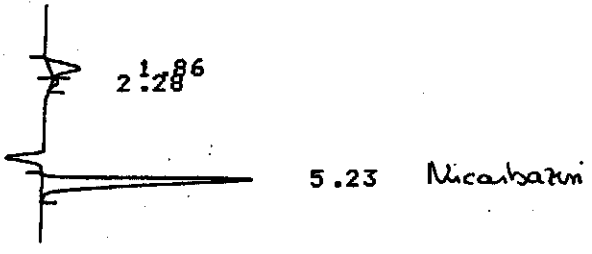
CH. 1 C.S 5.00 ATT 5 OFFS 10 11/22/00 11:31

Blank feed



CH. 1 C.S 5.00 ATT 5 OFFS 10 11/22/00 12:13

Standard 2,120 µg/ml



INJ NO. OF STD : 1 / 1 REP , 1st level

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 19

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: **Task 4 COLLABORATIVE STUDY**

Lab-name:

Contact person:

e-mail:
fax:
telephone:

Date of analysis:

Analyte: **NICARBAZIN**

Sample code	Unit	Result (mg/kg)
194508		0,4
194545		115
194553		1,3
194585		46
194601		120
194616		21
194627		260
194647		44
194655		270
194676		45

Sample	Unit	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture		11450	10870

Annex 4 - Questionnaire

Date(s) of analysis: .....١٥-١٢-٢٠٠٠.....

Dilution factor of the samples:

- Feed samples (specify for which feed samples): .....١.....
  .....
- Premixture: .....١٠.....

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ☒ Other: .....250 l.m.b....NUCLEOSIL.....120...5....C18.....
- Mobile phase:
  - ☒ As described in the method
  - ☐ Other: .....
- Flow-rate: .....١..... ml/min
- Injection volume: ..٢٠....µl
- Retention time of nicarbazin: ..6.8.. min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

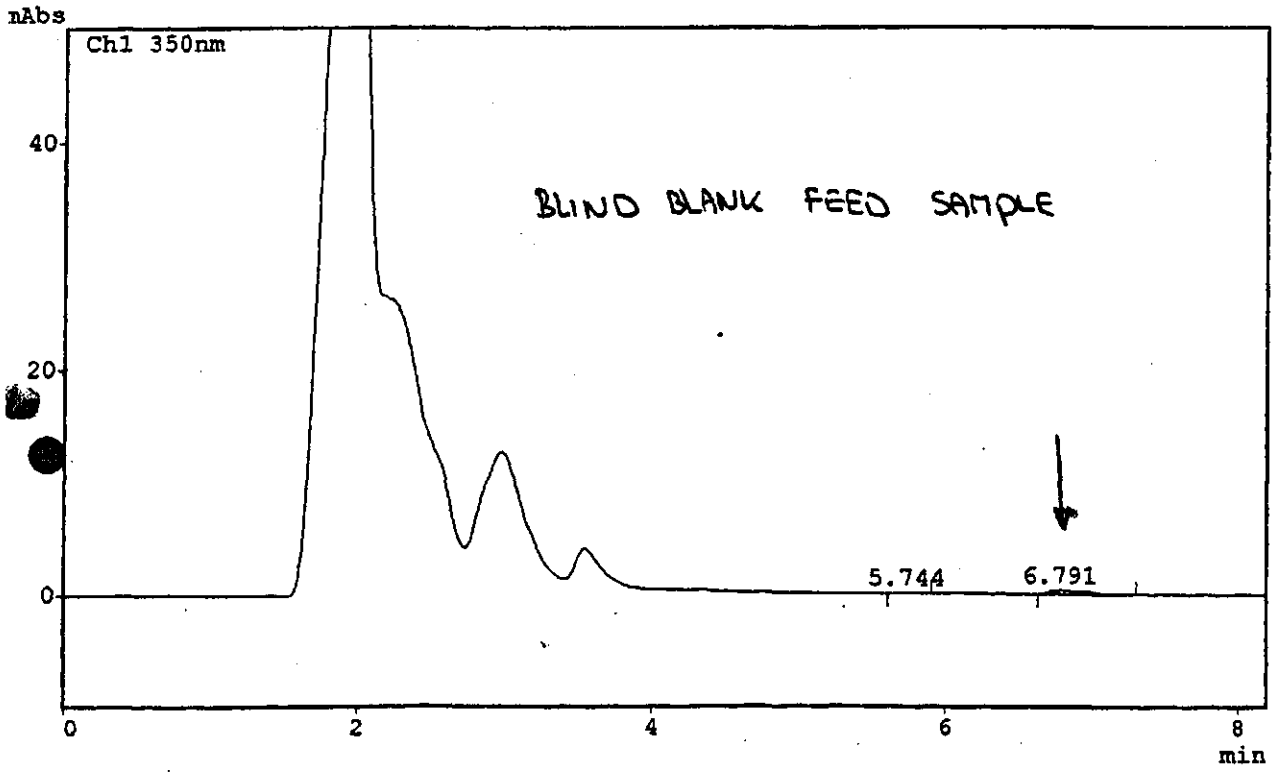
Recovery results:

- Percentage recovery: .١٠١.. %
- Single / duplicate determinations: ☒ single ☐ duplicate
- If duplicate, please give both percentages: ..... % and ..... %
- Spiking level: ...١٥٨... mg/kg

: 7
:
tion Factor: 1
e : Unknown
tector : SPD-M6A
erator :

19

* Chromatogram ***



* Peak Report ***

NO	ChNO	TIME	AREA	MK	PURITY.UP	PURITY.DOWN	IDNO	CONC
2	1	6.791	5963		0.9906(0.8363)	0.9069(0.9280)	1	100.0000
			5963					100.0000

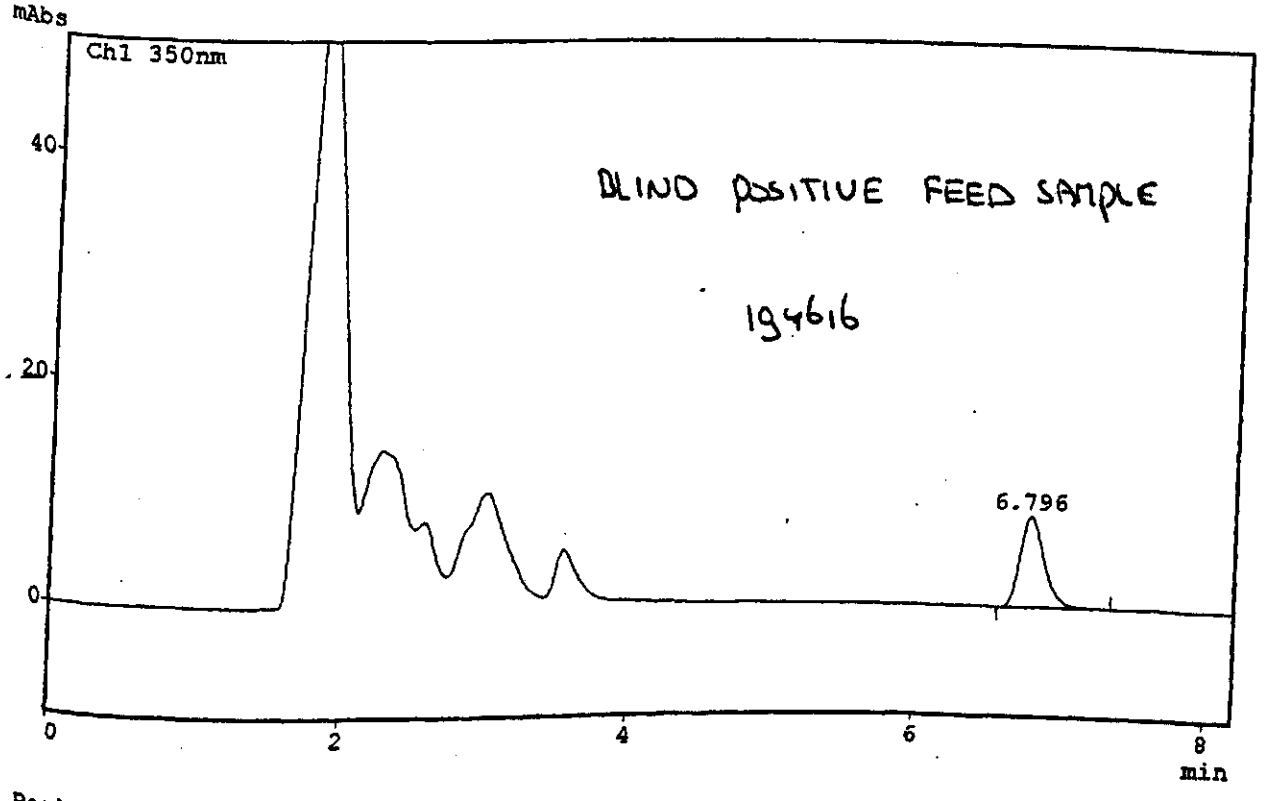
00/10/19 12:49:09

C10 SYS=1 REPORT.NO=18 DATA=NIC2.K18 00/10/19 13:06:41

:
: 10

tion Factor: 1
e : Unknown
tector : SPD-M6A
erator :

* Chromatogram ***



** Peak Report ***

UNO	ChNO	TIME	AREA	MK	PURITY.UP	PURITY.DOWN	IDNO	CONC
1	1	6.796	97312		0.9987(0.9962)	0.9968(0.9963)	1	100.0000
			97312					100.0000

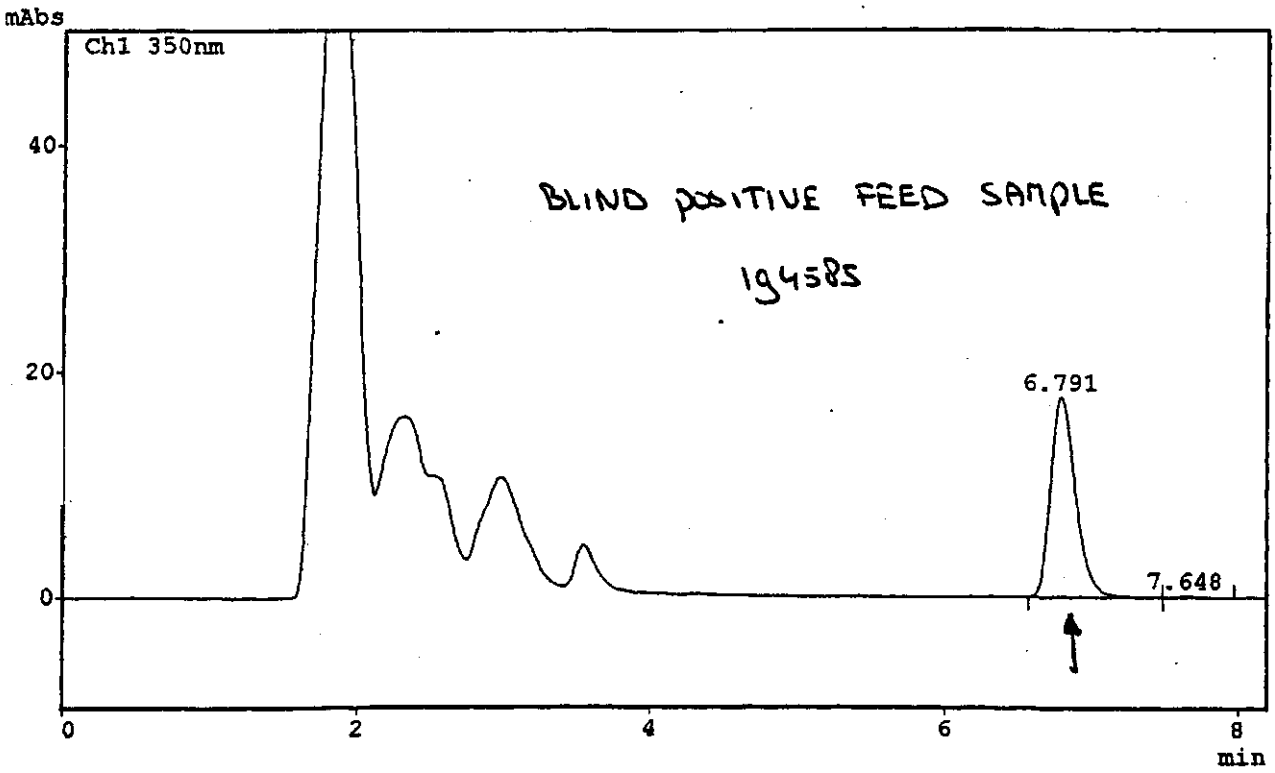
C10 SYS=1 REPORT.NO=16 DATA=N1C2.K16 00/10/19 12:49:27

: 8

tion Factor: 1
e : Unknown
tector : SPD-M6A
erator :

16

* Chromatogram ***



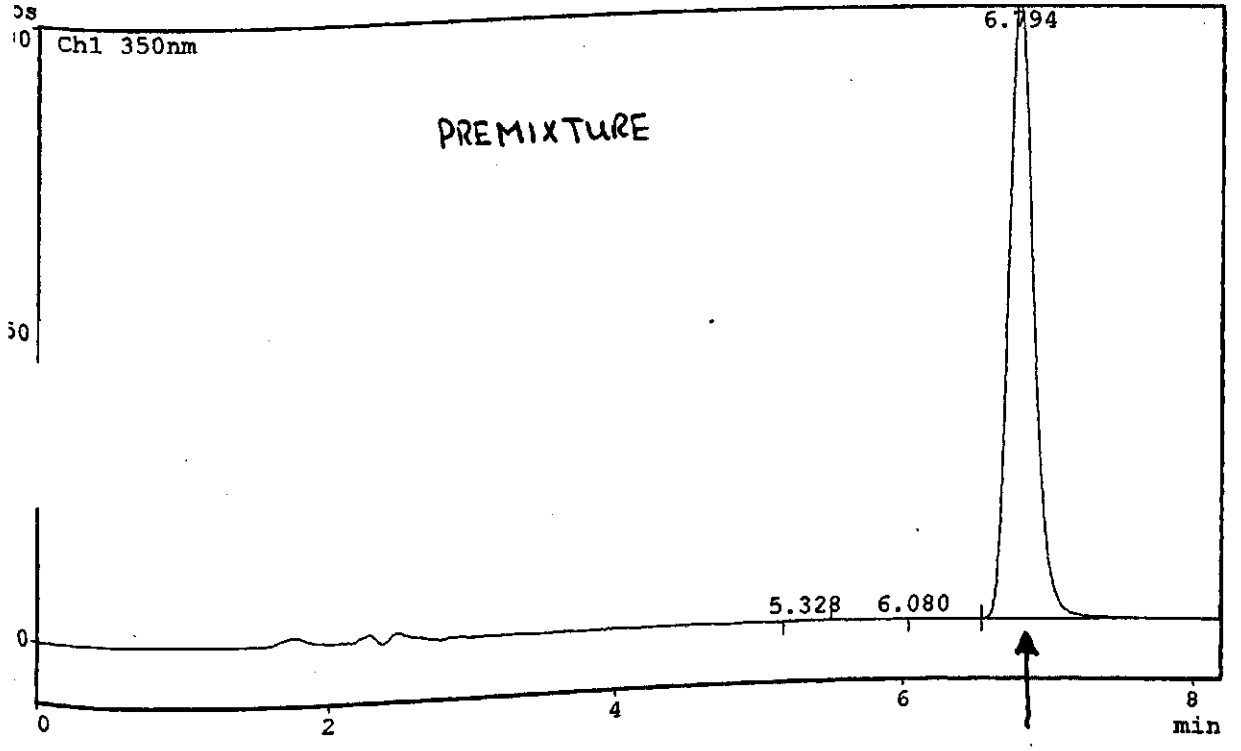
* Peak Report ***

NO	ChNO	TIME	AREA	MK	PURITY.UP	PURITY.DOWN	IDNO	CONC
1	1	6.791	211226		0.9989(0.9992)	0.7544(0.9973)	1	99.0990
2	1	7.648	1921		0.9607(0.0000)	0.8912(0.4900)	1	0.9010
			213147					100.0000

:
: 4
ion Factor: 1
: Unknown
tor : SPD-M6A
ator :

19

Chromatogram ***



Peak Report ***

ChNO	TIME	AREA	MK	PURITY.UP	PURITY.DOWN	IDNO	CONC
1	5.328	1232		0.9651(-0.152)	0.9965(0.8309)	3	
1	6.080	328		0.9426(0.0000)	0.2439(0.0000)	1	0.0269
1	6.794	1216393		1.0000(0.9999)	0.9977(0.9778)	1	99.9731
		1217953					100.0000

00/10/19 11:48:57

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 20

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: Task 4 COLLABORATIVE STUDY
Lab-name:
Contact person:

Date of analysis: november 23 2000
***Nicarbazin stock solution in DMF* ←
Analyte: NICARBAZIN

Sample code	Unit	Result (mg/kg)
204504		50,46
204571		26,92
204575		26,1
204583		273,53
204587		270,7
204634		53,5
204645		120,83
204683		121,27
204686		<LOQ
204688		<LOQ

Sample	Unit	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture		13693	13673

Nicarbazin stock solution prepared in DMF

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: Task 4 COLLABORATIVE STUDY
Lab-name:
Contact person:

e-mail:
fax:
telephone:

Date of analysis: November 25, 2000

Analyte: NICARBAZIN

Sample code	Unit	Result (mg/kg)
204504		75,79
204571		43,11
204575		41,94
204583		160,14
204587		351,27
204634		80,31
204645		159,51
204683		361,37
204686		neg
204688		neg

Sample	Unit	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture		21297	21633

Nicarbazin stock solution prepared according to the method

20

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Date(s) of analysis: 15/11/2000

Dilution factor of the samples:

- Feed samples (specify for which feed samples): 206583 5 kl 1:8 / 206587 27 kl 1:
- Premixture: 1:100

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ☒ Other: AppTech AppTime C18 250 x 46 5µm
- Mobile phase:
  - ☒ As described in the method
  - ☐ Other:
- Flow-rate: 1,000 ml/min
- Injection volume: 20 µl
- Retention time of nicarbazin: 7.09 min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

Recovery results:

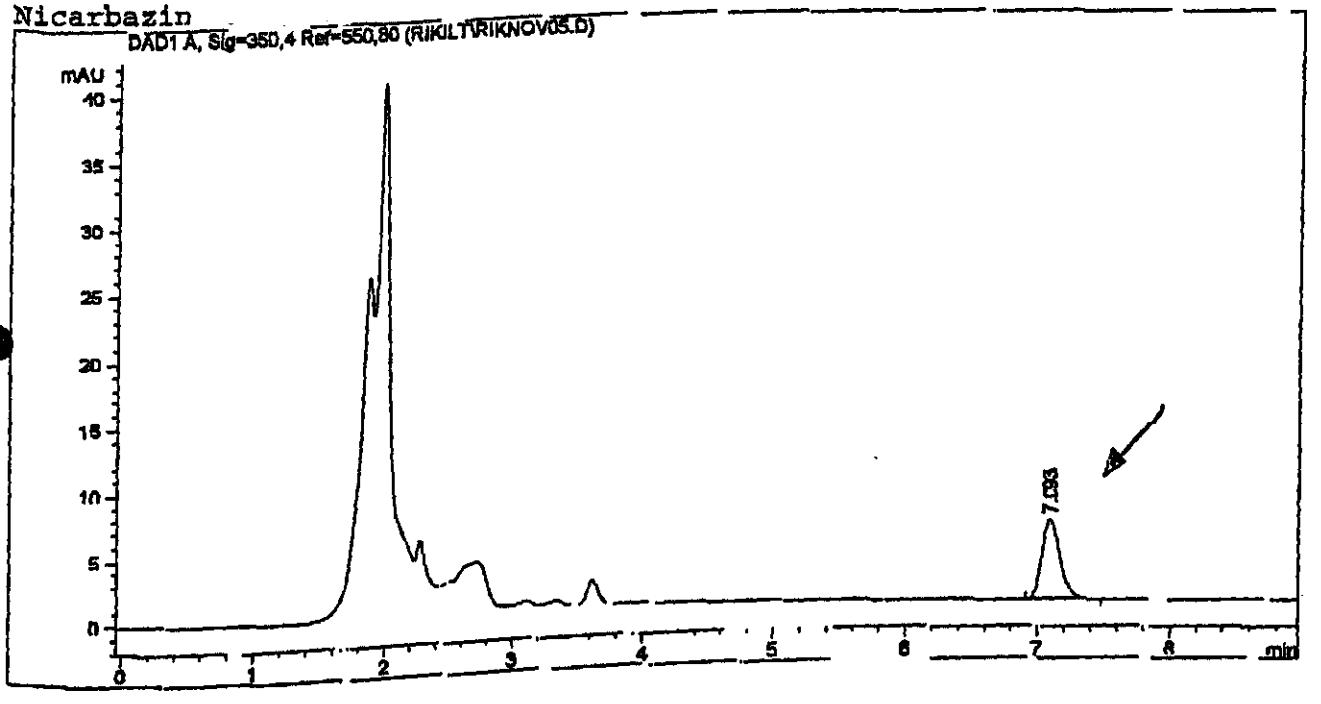
- Percentage recovery: 108 %
- Single / duplicate determinations: ☐ single ☒ duplicate
- If duplicate, please give both percentages: 107,7% and 108,1%
- Spiking level: 118 mg/kg

Injection Date : 11/25/2000 2:17:50 PM
Sample Name : 204571 54
Acq. Operator : ea

Seq. Line : 5
Vial : 5
Inj : 1
Inj Volume : 20 ul

Acq. Method : C:\HPCHEM\1\METHODS\RIKILT.M
Last changed : 11/24/2000 10:51:02 AM by ea
Analysis Method : C:\HPCHEM\1\METHODS\RIKILTLO.M
Last changed : 11/27/2000 12:45:36 PM by ea
(modified after loading)

Nicarbazin



External Standard Report

Sorted By : Signal
Calib. Data Modified : 11/22/2000 11:35:18 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=350,4 Ref=550,80

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
7.093	BB	58.88486	1.85059e-2	1.08972		Nicarbazin
				1.08972		

Totals :

Results obtained with enhanced integrator!

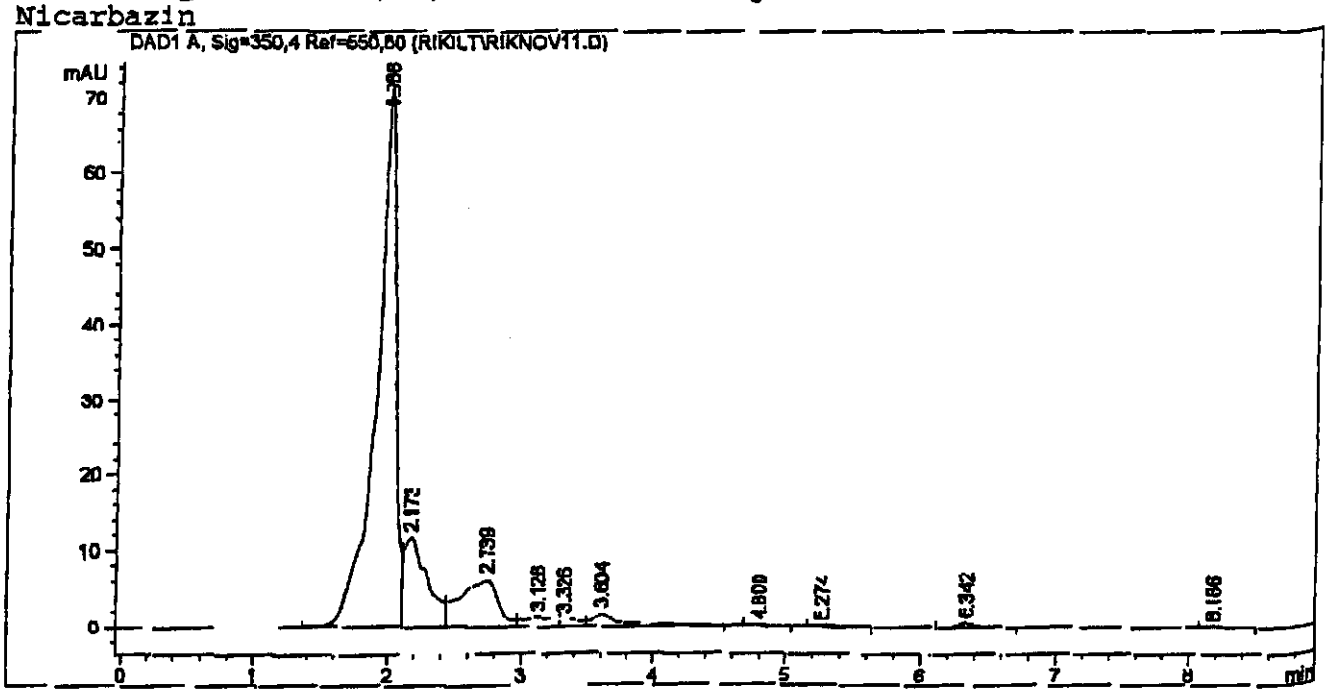
*** End of Report ***

Instrument 1 11/27/2000 12:46:37 PM ea

$$\frac{1.08\ \mu g/mL \cdot 100\ mL}{2.526\ gr} = 42.75\ \mu g/gr$$

Injection Date : 11/25/2000 3:25:20 PM Seq. Line : 11
Sample Name : 204686 75 Vial : 11
Acq. Operator : ea Inj : 1
Inj Volume : 20 ul

Acq. Method : C:\HPCHEM\1\METHODS\RIKILT.M
Last changed : 11/24/2000 10:51:02 AM by ea
Analysis Method : C:\HPCHEM\1\METHODS\RIKILTHI.M
Last changed : 11/22/2000 4:48:33 PM by ea
Nicarbazin



External Standard Report

Sorted By : Signal
Calib. Data Modified : 11/22/2000 4:45:48 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=350,4 Ref=550,80

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
7.142			-	-		NICARBAZIN

Totals : 0.00000

Results obtained with enhanced integrator!
1 Warnings or Errors :

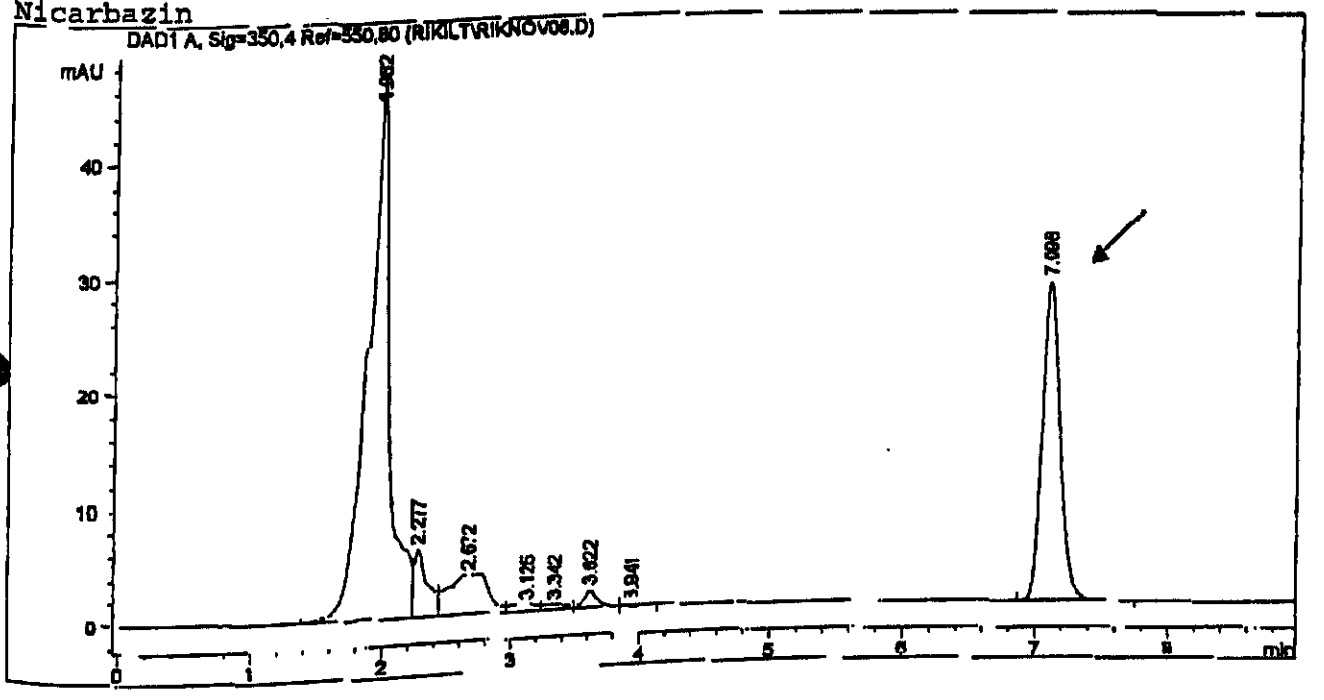
Warning : Calibrated compound(s) not found

20

Injection Date : 11/25/2000 2:29:06 PM
Sample Name : 204683 31
Acq. Operator : ea

Seq. Line : 6
Vial : 6
Inj : 1
Inj Volume : 20 ul

Acq. Method : C:\HPCHEM\1\METHODS\RIKILT.M
Last changed : 11/24/2000 10:51:02 AM by ea
Analysis Method : C:\HPCHEM\1\METHODS\RIKILTHI.M
Last changed : 11/22/2000 4:48:33 PM by ea
Nicarbazin



External Standard Report

Sorted By : Signal
Calib. Data Modified : 11/22/2000 4:45:48 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=350,4 Ref=550,80

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
7.098	BB	264.73093	1.52681e-2	4.04193		NICARBAZIN
Totals :				4.04193		

Results obtained with enhanced integrator!

*** End of Report ***

Instrument 1 11/27/2000 12:47:21 PM ea

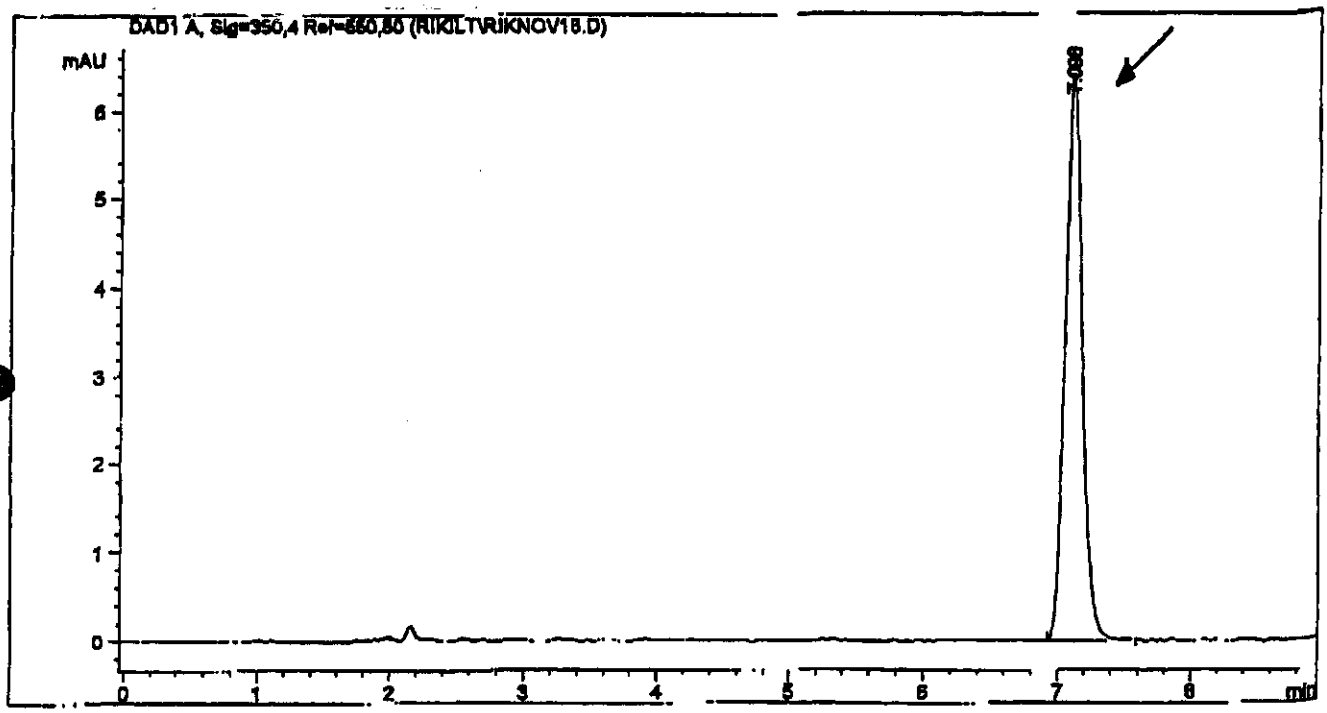
4.04 µg/mL . 100 mL = 160.0 µg/gr
2.524 gr

Data File C:\HPCHEM\1\DATA\RIKILT\RIKNOV18.D Sample Name: premixture

Injection Date	: 11/27/2000 12:20:21 PM	Seq. Line	: 1
Sample Name	: premixture dil 1:100	Vial	: 5
Acq. Operator	: ea	Inj	: 1
		Inj Volume	: 20 ul

2°

Acq. Method : C:\HPCHEM\1\METHODS\RIKILT.M
Last changed : 11/24/2000 10:51:02 AM by ea
Analysis Method : C:\HPCHEM\1\METHODS\RIKILTLO.M
Last changed : 11/22/2000 4:21:38 PM by ea



External Standard Report

Sorted By	:	Signal
Calib. Data Modified	:	11/22/2000 11:35:18 AM
Multiplier	:	1.0000
Dilution	:	1.0000

Signal 1: DAD1 A, Sig=350,4 Ref=550,80

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
7.098	BB	57.69089	1.85058e-2	1.06762		Nicarbazin

Totals : 1.06762

Results obtained with enhanced integrator!

*** End of Report ***

2.12% 0.501 g = 1,98 mg % corrected

$\frac{1,0676 \times 100 \times 50}{0,501}$ = 1064,41 ppb 20%

= 19693,92

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 21

CANFAS

Development and Validation of HPLC-methods for the official control Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2

Subtitle: **Task 4 COLLABORATIVE STUDY**

Lab-name:
Contact person:
e-mail:
fax:
telephone:

Date of analysis:

Analyte: **NICARBAZIN**

	Unit	Result (mg/kg)
Sample code		
214503		251,6
214516		21,8
214526		0,0 N.D.
214531		52
214577		21
214580		0,0 N.D.
214606		118,8
214663		44,6
214673		122
214684		252,2

Unit / Sample	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture	9342	9461

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Date(s) of analysis: 2-11-2000 / 1-12-2000

Dilution factor of the samples:

- Feed samples (specify for which feed samples): .....
- Premixture: 1:25

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ☒ Other: SUPELCOSIL LC18 25cm × 4,6 mm (5 µm) WITH SUPELGUARD LC18
- Mobile phase:
  - ☒ As described in the method
  - ☐ Other: .....
- Flow-rate: 1 ml/min
- Injection volume: 20 µl
- Retention time of nicarbazin: 7.3 min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

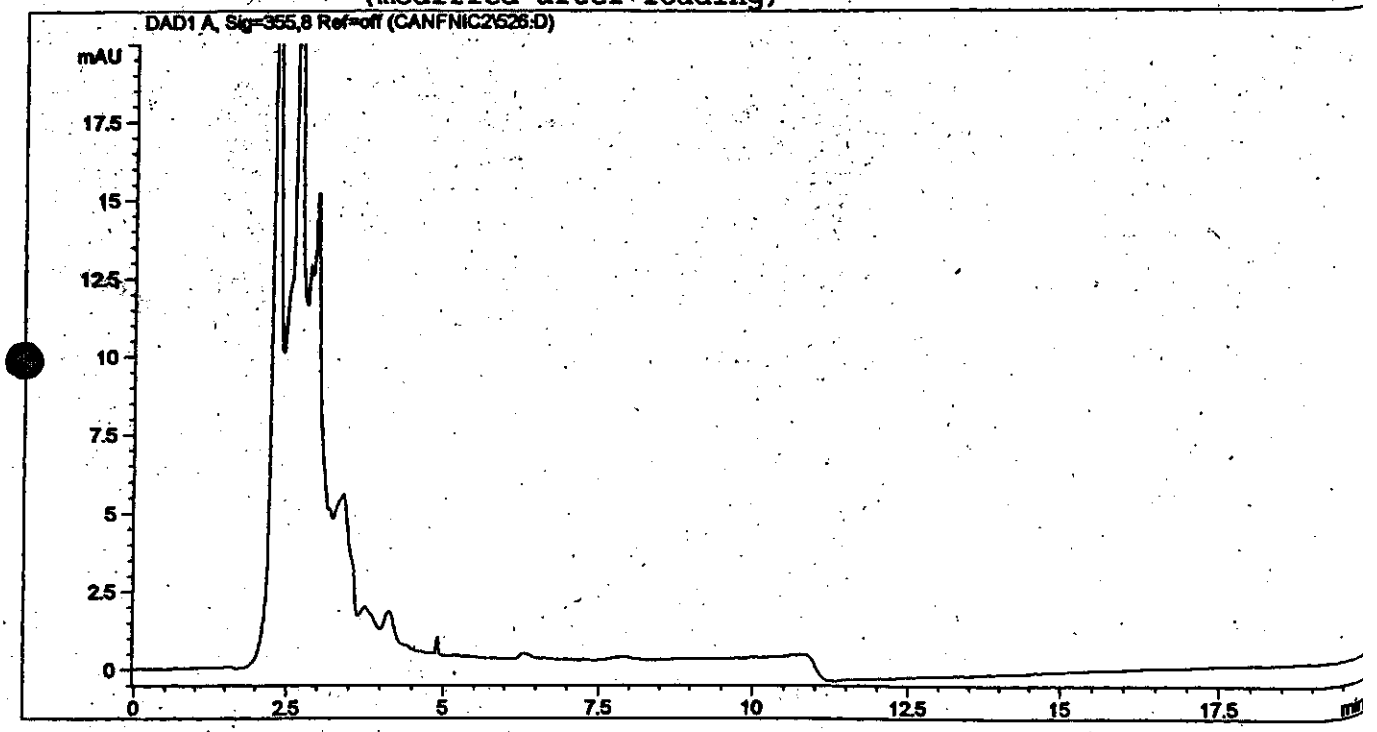
Recovery results:

- Percentage recovery: 100 %
- Single / duplicate determinations: ☒ single ☐ duplicate
- If duplicate, please give both percentages: ..... % and ..... %
- Spiking level: 100 mg/kg

ta File C:\HPCHEM\2\CROMAT\CANFNIC2\526.D Sample Name: 526

Injection Date : 02/11/2000 19.16.18 Seq. Line : 12
Sample Name : 526 Vial : 9
Acq. Operator : Inj : 1
Inj Volume : 20 µl

Acq. Method : C:\HPCHEM\2\METODI\CANFNICA.M
Last changed : 07/07/2000 15.25.45 by
Analysis Method : C:\HPCHEM\2\METODI\CANFNICA.M
Last changed : 03/11/2000 14.12.51
(modified after loading)



External Standard Report

Sorted By : Signal
Calib. Data Modified : 03/11/2000 13.54.31
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=355,8 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng/inj]	Grp	Name
7.328		-	-	-		NICA-nicarbazina

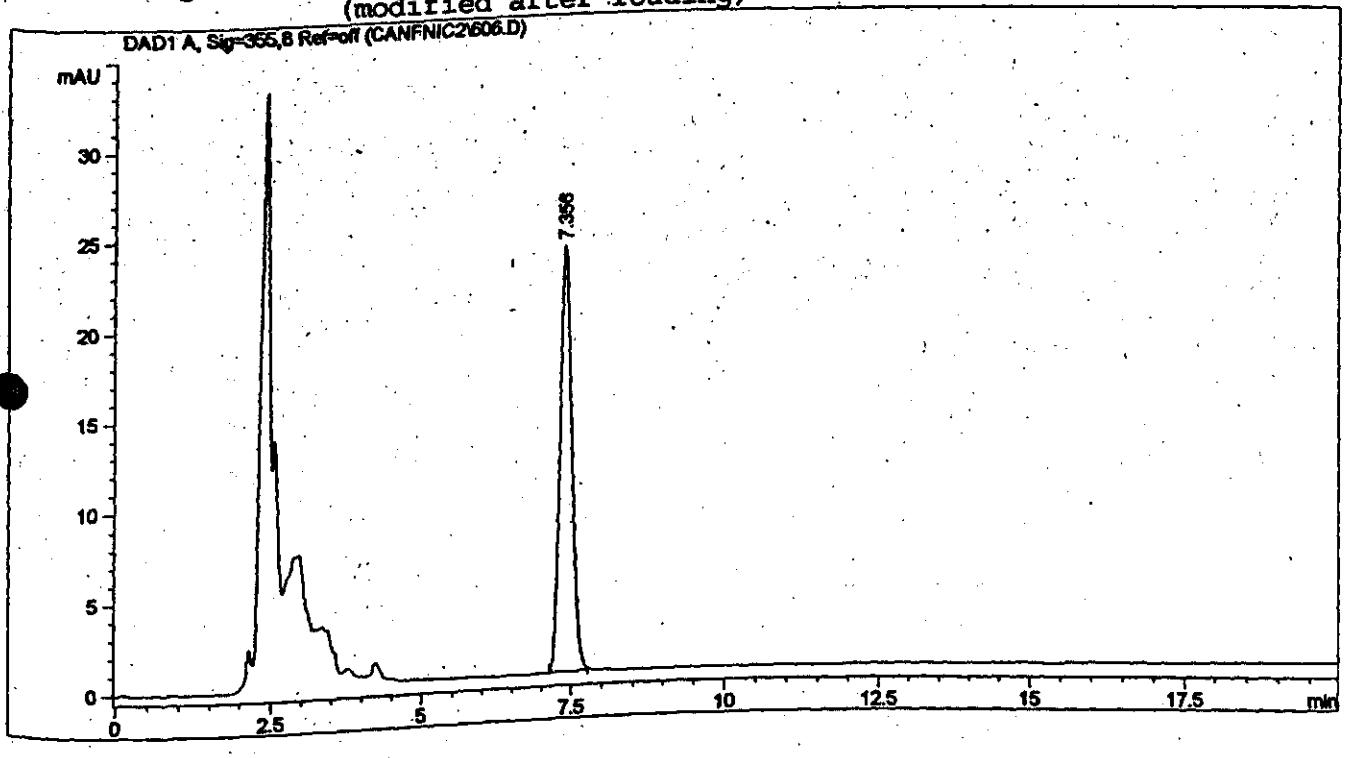
Totals : 0.00000

Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Data File C:\HPCHEM\2\CROMAT\CANFNIC2\606.D

Injection Date	: 02/11/2000 21.45.40	Seq. Line	: 19
Sample Name	: 606	Vial	: 14
Acq. Operator	:	Inj	: 1
		Inj Volume	: 20 µl
Acq. Method	: C:\HPCHEM\2\METODI\CANFNICA.M		
Last changed	: 07/07/2000 15.25.45 by		
Analysis Method	: C:\HPCHEM\2\METODI\CANFNICA.M		
Last changed	: 03/11/2000 14.37.04 (modified after loading)		



External Standard Report

Sorted By	:	Signal
Calib. Data Modified	:	03/11/2000 13.54.31
Multiplier	:	1.0000
Dilution	:	1.0000

Signal 1: DAD1 A, Sig=355,8 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng/inj]	Grp	Name
7.356	BB	329.56863	1.80129e-1	59.36477		NICA-nicarbazina
Totals :				59.36477		

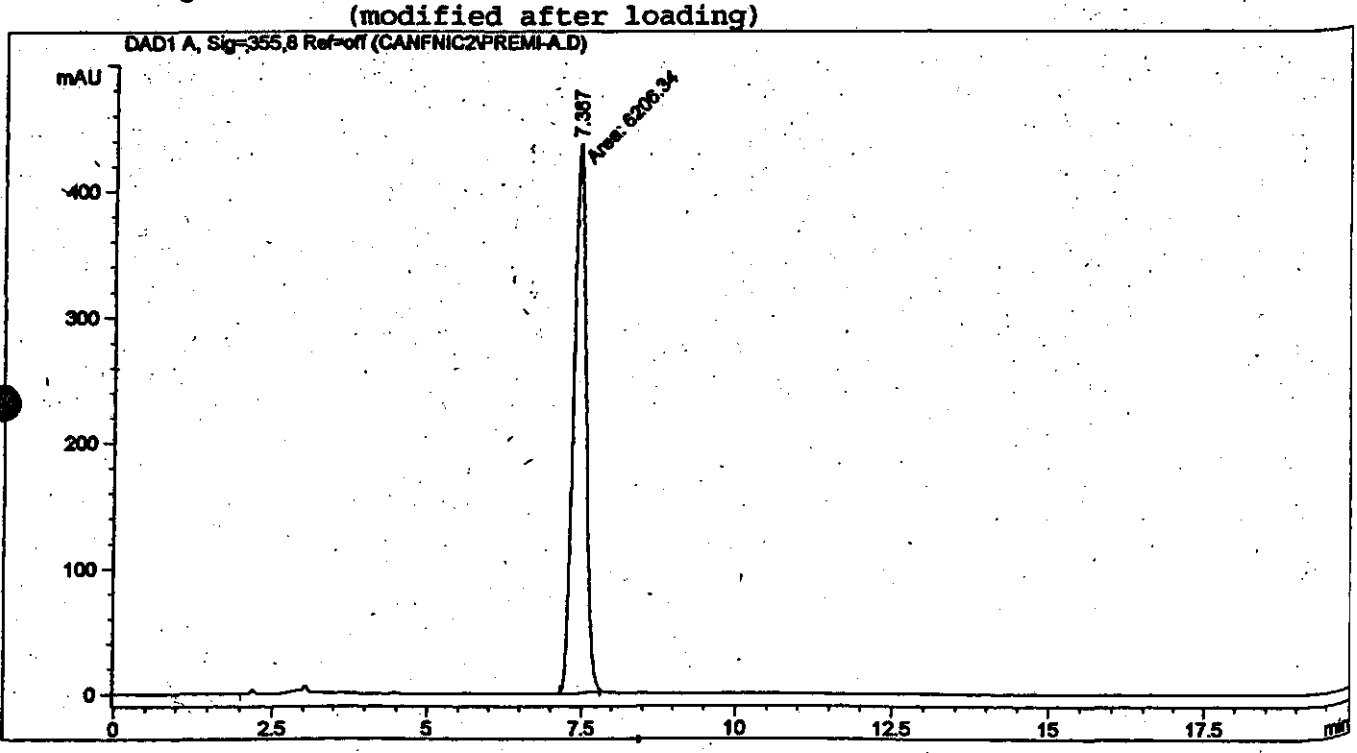
Results obtained with enhanced integrator!

Diode Array 03/11/2000 14.37.07

21

Injection Date : 02/11/2000 22.28.16 Seq. Line : 21
Sample Name : premix-a Vial : 16
Acq. Operator : Inj : 1
Inj Volume : 20 µl

Acq. Method : C:\HPCHEM\2\METODI\CANFNICA.M
Last changed : 07/07/2000 15.25.45 by
Analysis Method : C:\HPCHEM\2\METODI\CANFNICA.M
Last changed : 03/11/2000 14.51.15
(modified after loading)



External Standard Report

Sorted By : Signal
Calib. Data Modified : 03/11/2000 13.54.31
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=355,8 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng/inj]	Grp	Name
7.387	MM	~~6206.33936~~ 905.05	~~1.78428e-1~~ 1.08711e-1	~~1107.38280~~ 42.79		NICA-nicarbazina
Totals :				1107.38280		

Results obtained with enhanced integrator!

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 23

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: Task 4 COLLABORATIVE STUDY
Lab-name:
Contact person:

e-mail:
fax:
telephone:

Date of analysis:

Analyte: NICARBAZIN

	Unit	Result (mg/kg)
Sample code		
234510		< 5,93
234524		44,25
234525		29,91
234547		241,03
234552		21,08
234582		100,32
234596		< 5,93
234598		118,19
234607		39,73
234652		260,78

Unit / Sample	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture	1,06	0,99

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 24

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: **Task 4 COLLABORATIVE STUDY**
Lab-name:
Contact person: e-mail:
fax:
telephone:
Date of analysis:

Analyte: **NICARBAZIN**

Sample code	Unit	Result (mg/kg)
244505		48,4
244513		111,8
244528		108,8
244533		blank
244550		22,9
244578		264,8
244586		22,6
244619		blank
244622		265,3
244630		50,2

Sample	Unit	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture		12728	12968

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Date(s) of analysis: 20th october 2000

Dilution factor of the samples:

- Feed samples (specify for which feed samples): .....
- Premixture: 10 x

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ☒ Other: 250 mm x 4.6 mm C18 5 µm
- Mobile phase:
  - ☒ As described in the method
  - ☐ Other: .....
- Flow-rate: 1 ml/min
- Injection volume: 20 µl
- Retention time of nicarbazin: ~6.3 min

Chromatograms: Please include representative chromatograms of:

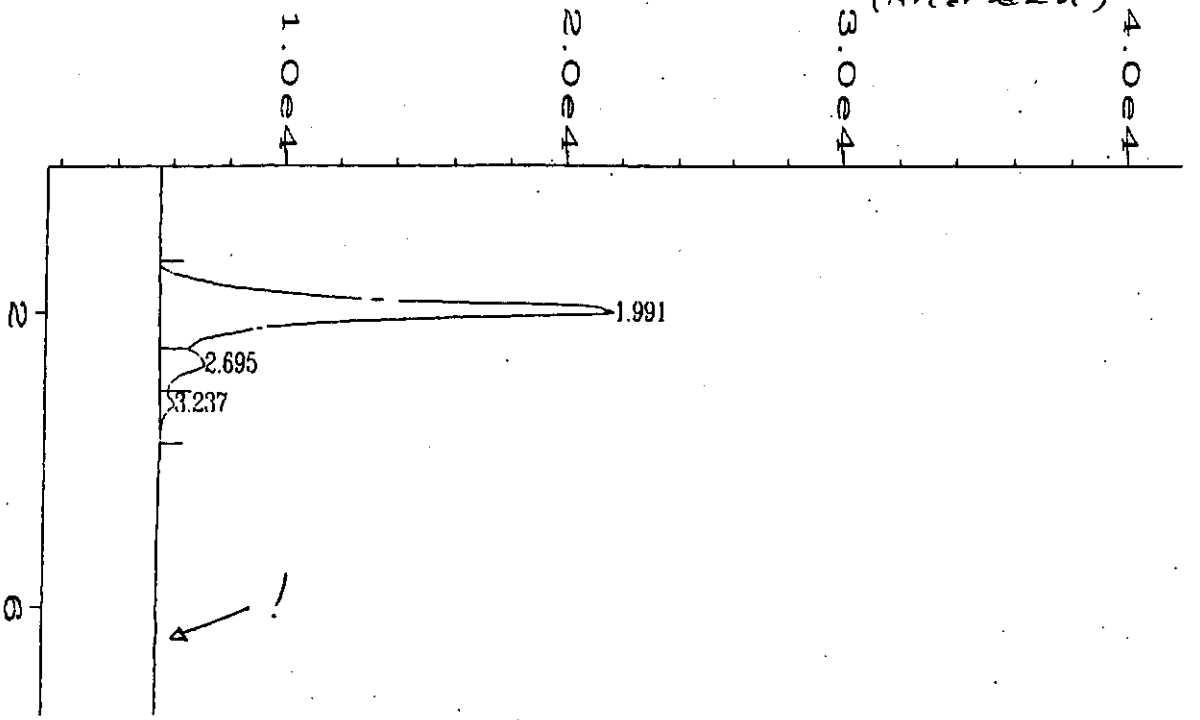
- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

Recovery results:

- Percentage recovery: 100 % (average)
- Single / duplicate determinations: ☐ single ☒ duplicate
  If duplicate, please give both percentages: 99 % and 101 %
  Spiking level: 100 mg/kg

(Nicarbazin)



Area Percent Report

Data File Name : C:\HPCHEM\1\DATA\DACS\CANFAS19.D
Operator :
Instrument : ISOCRATIC
Sample Name :
Run Time Bar Code:
Acquired on : 20 Oct 90 05:13 PM
Report created on: 20 Oct 90 05:27 PM
Sample Info :

Page Number : 1
Vial Number :
Injection Number :
Sequence Line :
Instrument Method: FURAN.MTH
Analysis Method : FURAN.MTH

Sig. 1 in C:\HPCHEM\1\DATA\DACS\CANFAS19.D

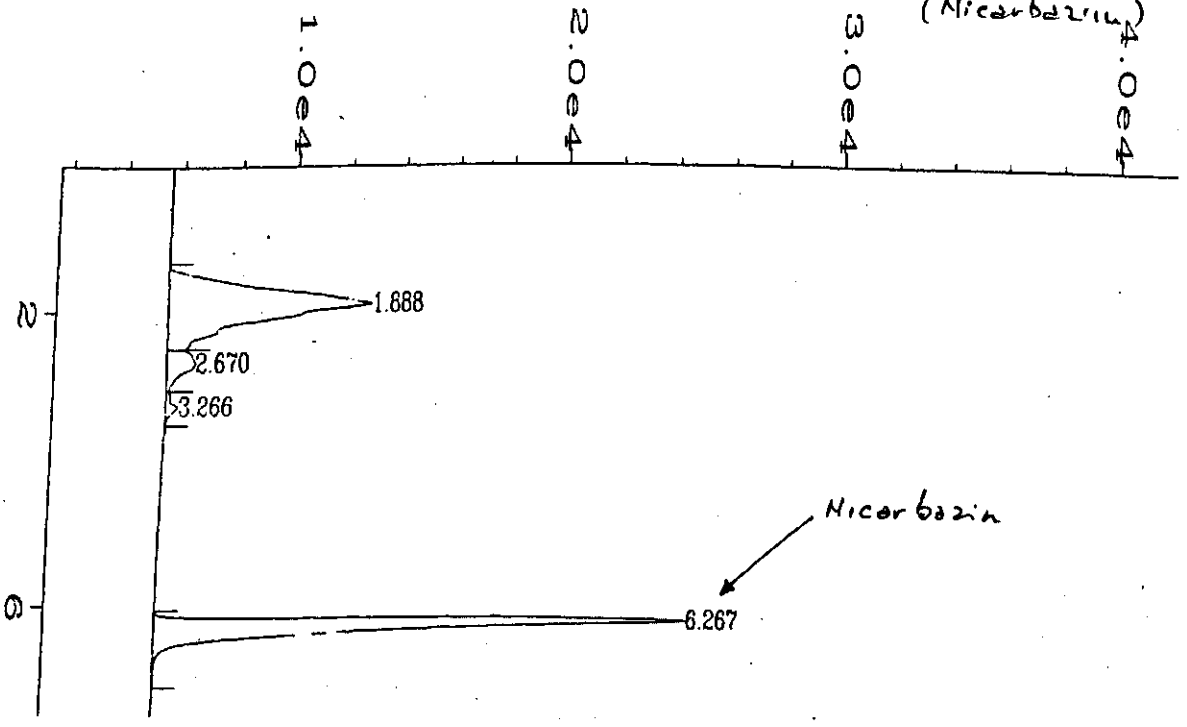
Pk#	Ret Time	Area	Height	Type	Width	Area %
1	1.991	351170	16171	BV	0.278	89.3179
2	2.695	33941	1570	VV	0.291	8.6328
3	3.237	8057	498	VB	0.216	2.0493

Total area = 393168

Sample 6

Sample # 244578
(Nicarbazin)

24

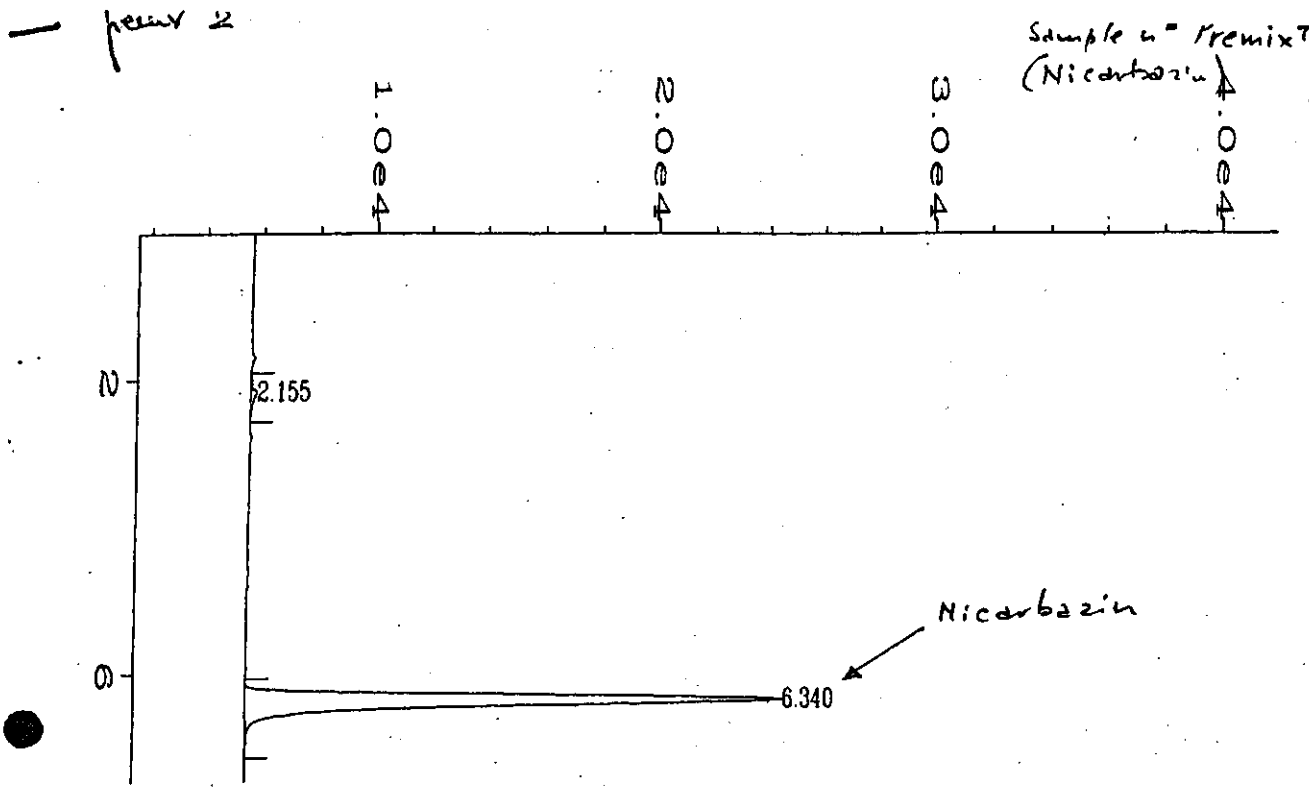


External Standard Report

Data File Name	: C:\HPCHEM\1\DATA\DACS\CANFAS17.D		
Operator	:	Page Number	: 1
Instrument	: ISOCRATIC	Vial Number	:
Sample Name	:	Injection Number	:
Run Time Bar Code:		Sequence Line	:
Acquired on	: 20 Oct 90 04:58 PM	Instrument Method:	FURAN.MTH
Report Created on:	20 Oct 90 05:06 PM	Analysis Method	: FURAN.MTH
Last Recalib on	: 20 Oct 90 04:08 PM	Sample Amount	: 0
Multiplier	: 1	ISTD Amount	:

Signal 1 in C:\HPCHEM\1\DATA\DACS\CANFAS17.D

Ret Time	Area	Type	Width	Ref#	ng/ul	Name
6.267	204803	BB	0.161	1	6.622	nicarbazin



External Standard Report

Data File Name : C:\HPCHEM\1\DATA\DACS\CANFAS28.D
Operator :
Instrument : ISOCRATIC
Sample Name :
Run Time Bar Code:
Acquired on : 20 Oct 90 06:27 PM
Report Created on: 20 Oct 90 06:37 PM
Last Recalib on : 20 Oct 90 04:08 PM
Multiplier : 1

Page Number : 1
Vial Number :
Injection Number :
Sequence Line :
Instrument Method: FURAN.MTH
Analysis Method : FURAN.MTH
Sample Amount : 0
ISTD Amount :

Sig 1 in C:\HPCHEM\1\DATA\DACS\CANFAS28.D

Ret Time	Area	Type	Width	Ref#	ng/ul	Name
6.340	200550	BB	0.160	1	6.484	nicarbazin

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 25

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: Task 4 COLLABORATIVE STUDY
Lab-name:
Contact person:

e-mail:
fax:
telephone:

Date of analysis:

Analyte: NICARBAZIN

Sample code	Unit	Result (mg/kg)
254527		nd
254536		20,01
254540		100,01
254551		nd
254554		285,90
254602		209,90
254658		36,35
254659		100,00
254685		20,00
254689		35,91

Sample	Unit	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture		13900	13900

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Date(s) of analysis: 12-11-2000

Dilution factor of the samples:

- Feed samples (specify for which feed samples): .....
- Premixture: 1:100

Chromatographic conditions:

- Column:
  - ☒ As described in the method
  - ☐ Other: .....
- Mobile phase:
  - ☒ As described in the method
  - ☐ Other: .....
- Flow-rate: 1 ml/min
- Injection volume: 20 µl
- Retention time of nicarbazin: 5.65 min

Chromatograms: Please include representative chromatograms of:

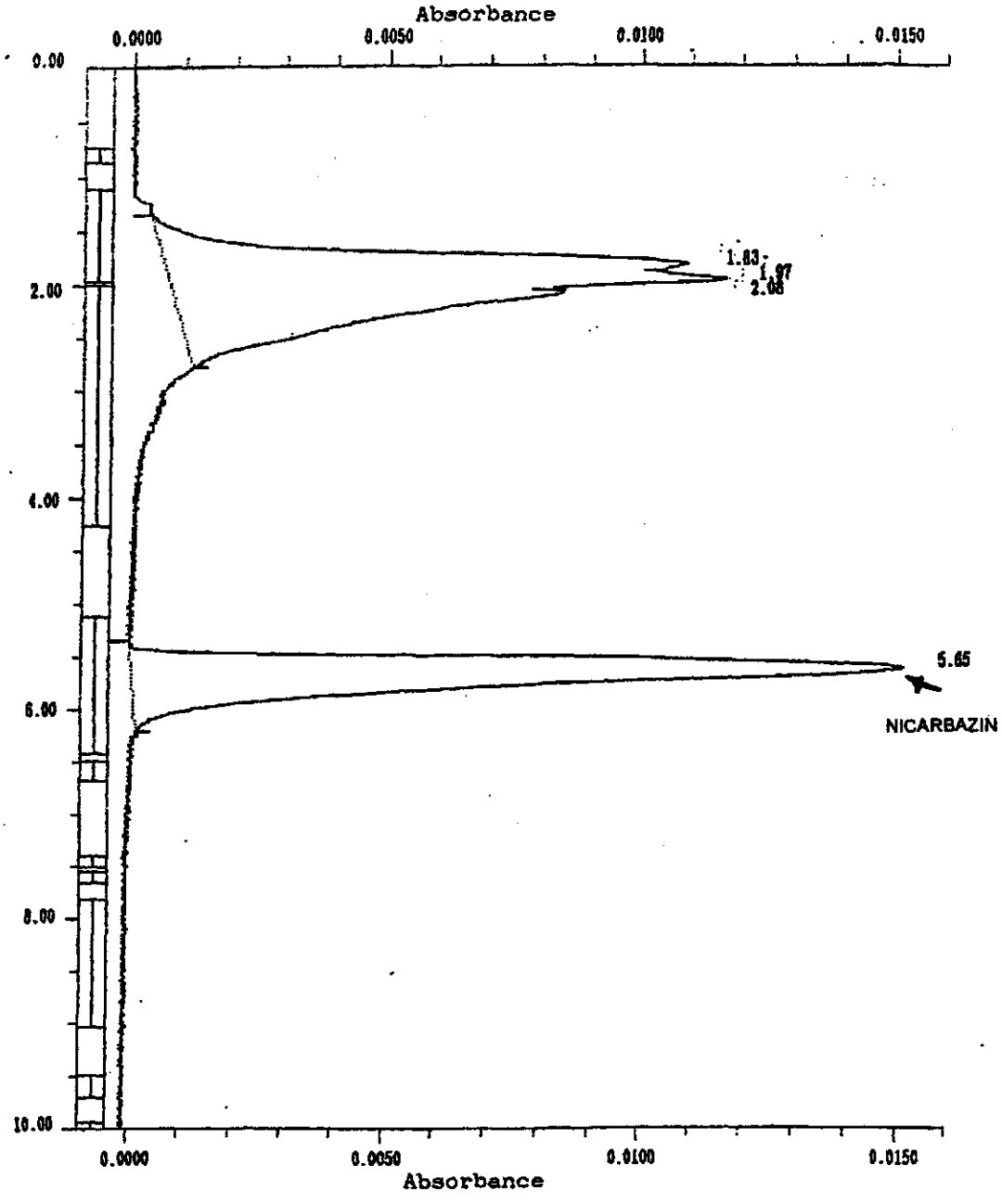
- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

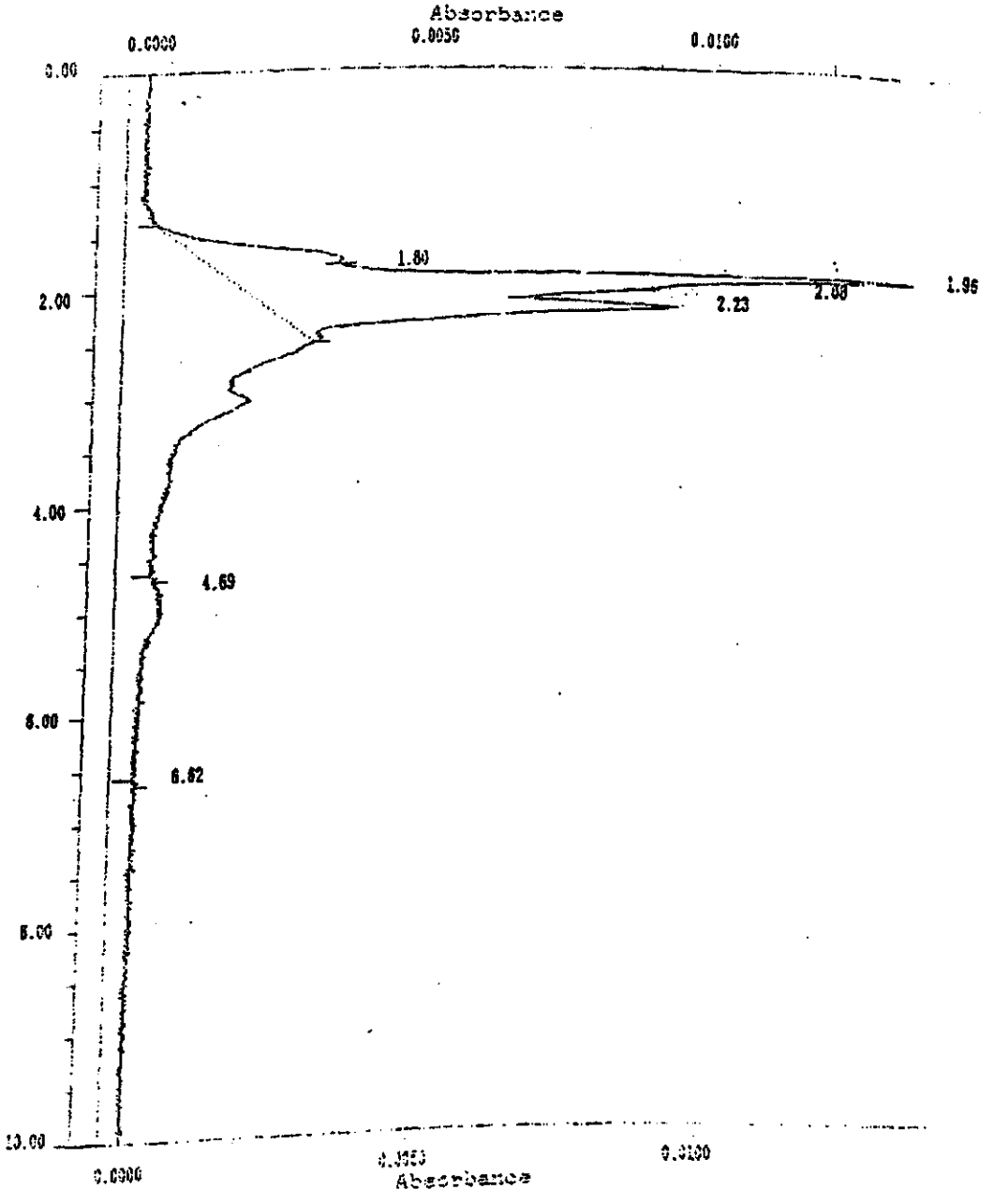
Recovery results:

- Percentage recovery: 91 %
- Single / duplicate determinations: ☒ single ☐ duplicate
- If duplicate, please give both percentages: ..... % and ..... %
- Spiking level: 20 mg/kg

Chromatogram for Nicarbazin study

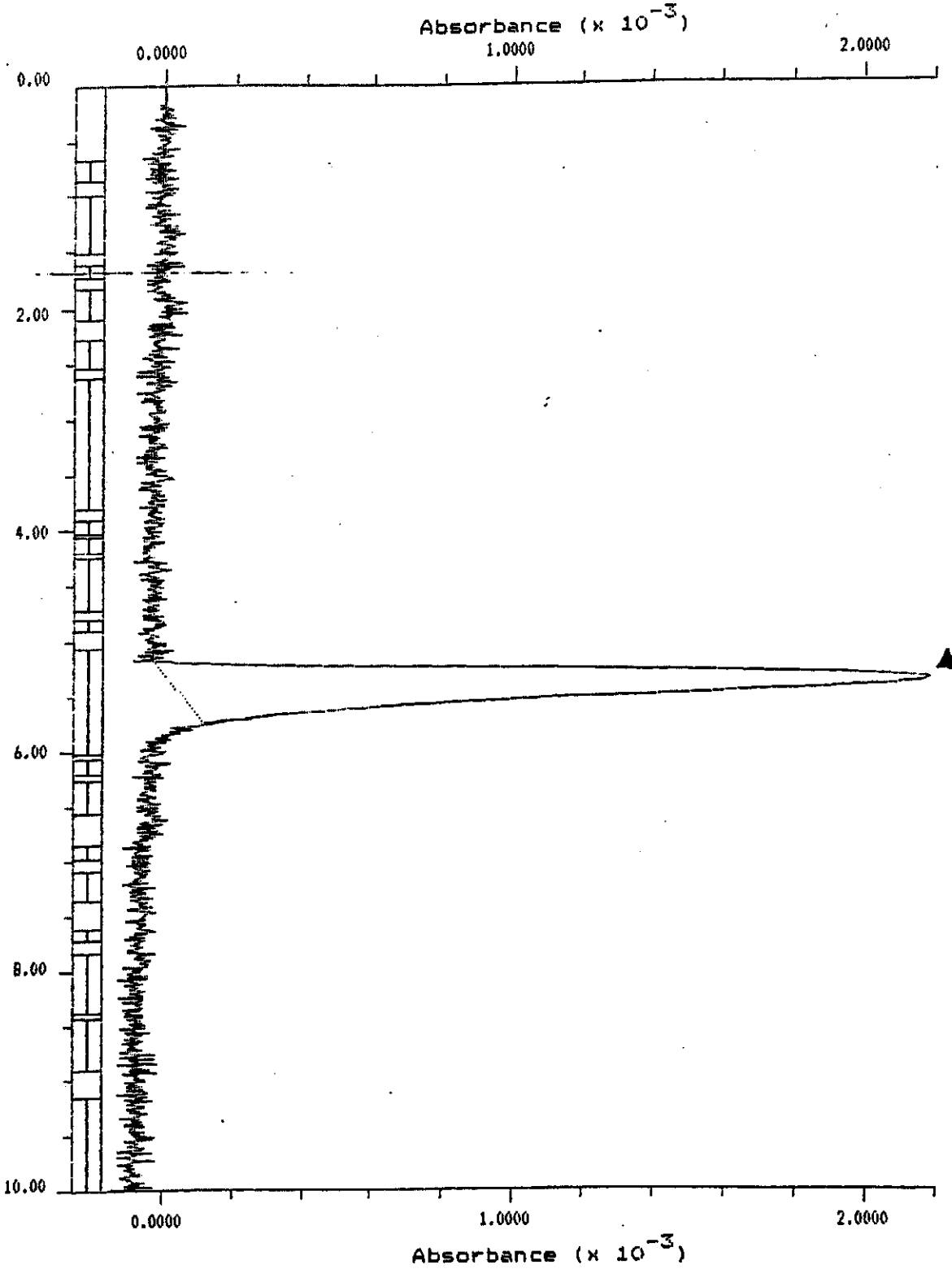


Feed n° 254554



Feed n° 254527 (Blank)

Chromatogram for Nicarbazin study



Premixture

Chromatogram for Nicarbazin study

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 26

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: **Task 4 COLLABORATIVE STUDY**
Lab-name:
Contact person:

e-mail:
fax:
telephone:

Date of analysis:

Analyte: **NICARBAZIN**

Sample code	Unit	Result (mg/kg)
264518		0
264529		23,8
264537		53,3
264544		115
264555		342
264566		0
264603		20,4
264648		359
264664		45,1
264679		128

Sample	Unit	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture		11833	13462

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Date(s) of analysis: 23/11/00

Dilution factor of the samples:

- Feed samples (specify for which feed samples): 26455, 26648 x 2
- Premixture: x20

Chromatographic conditions:

- Column:
  - ☐ As described in the method LUNA
  - ☐ Other: ~~SAXCSCB~~ ODS 2 250 x 4.6mm
- Mobile phase:
  - ☑ As described in the method
  - ☐ Other: .....
- Flow-rate: 1.0 ml/min
- Injection volume: 20 µl
- Retention time of nicarbazin: 7.9 min

Chromatograms: Please include representative chromatograms of:

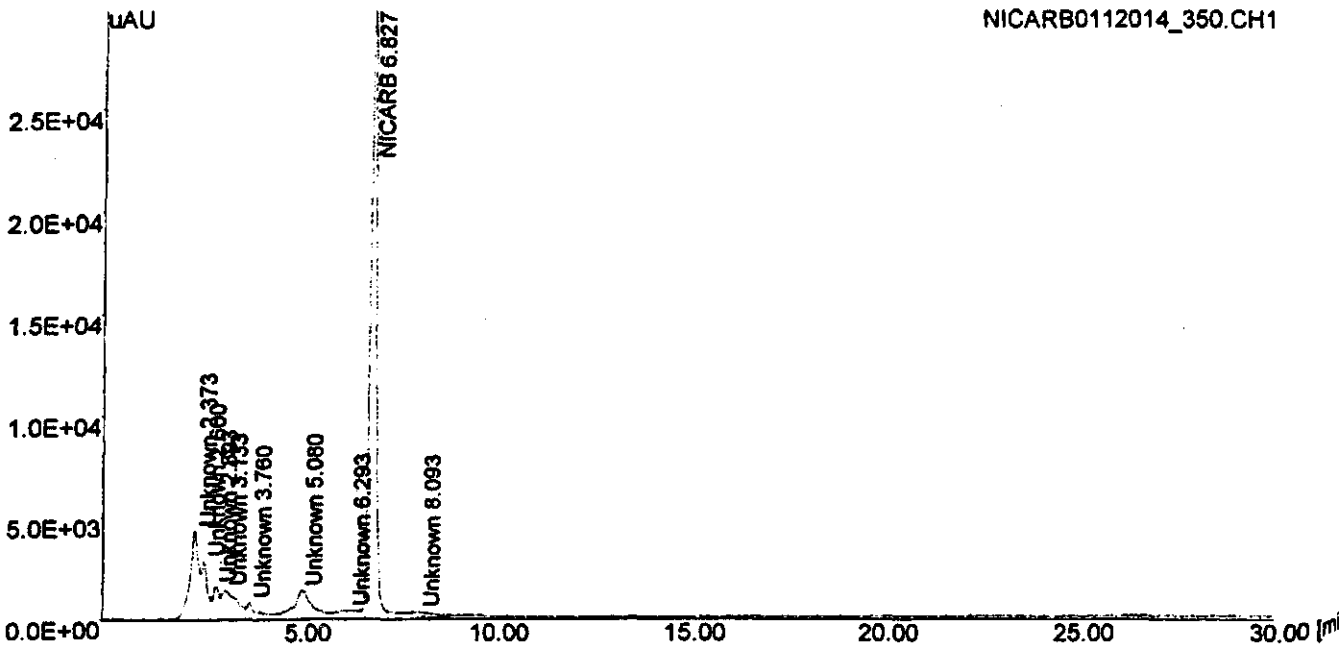
- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

Recovery results:

- Percentage recovery: 101 %
- Single / duplicate determinations: ☑ single ☐ duplicate
- If duplicate, please give both percentages: ..... % and ..... %
- Spiking level: 100 mg/kg

Chromatogram BLIND POSITIVE



File name : NICARB0112014_350.CH1

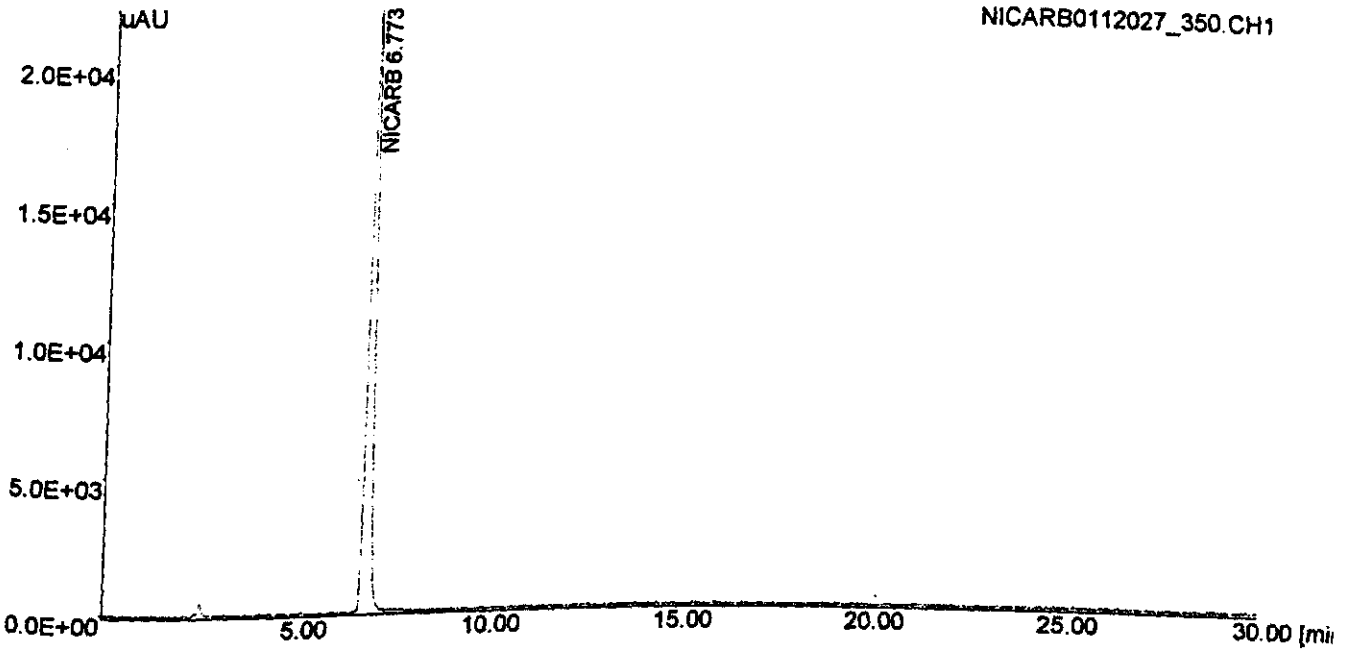
Info :
a3009168
Wavelength = 350 [nm]
Tacc [Sec] = 0.80 Wacc [nm] = 4.0
Autozero [min] : 0.00

Vial # = 7 Rack # = 0
Injection Date : 1-Dec-2000 18:42:52
Curr. Date : 19-Dec-2000 12:48:46
User : KL
Group : NICARB
Control Method :

#	Name	RT	Height[uAU]	Area[uAU.Sec]	%Area
1		2.373	4396	65179.292	11.84
2		2.600	2907	32034.534	5.82
3		2.893	1609	18418.806	3.35
4		3.133	1427	36406.442	6.61
5		3.760	842	10778.100	1.96
6		5.080	1366	47420.783	8.61
7		6.293	377	11153.854	2.03
8	NICARB	6.827	29779	322960.570	58.66
9		8.093	279	6189.412	1.12

Total Area of Peak = 550541.792 [uAU.Sec]

Chromatogram PREMIX



File name : NICARB0112027_350.CH1

Info :
a3009174a
Wavelength = 350 [nm]
Tacc [Sec] = 0.80 Wacc [nm] = 4.0
Autozero [min] : 0.00

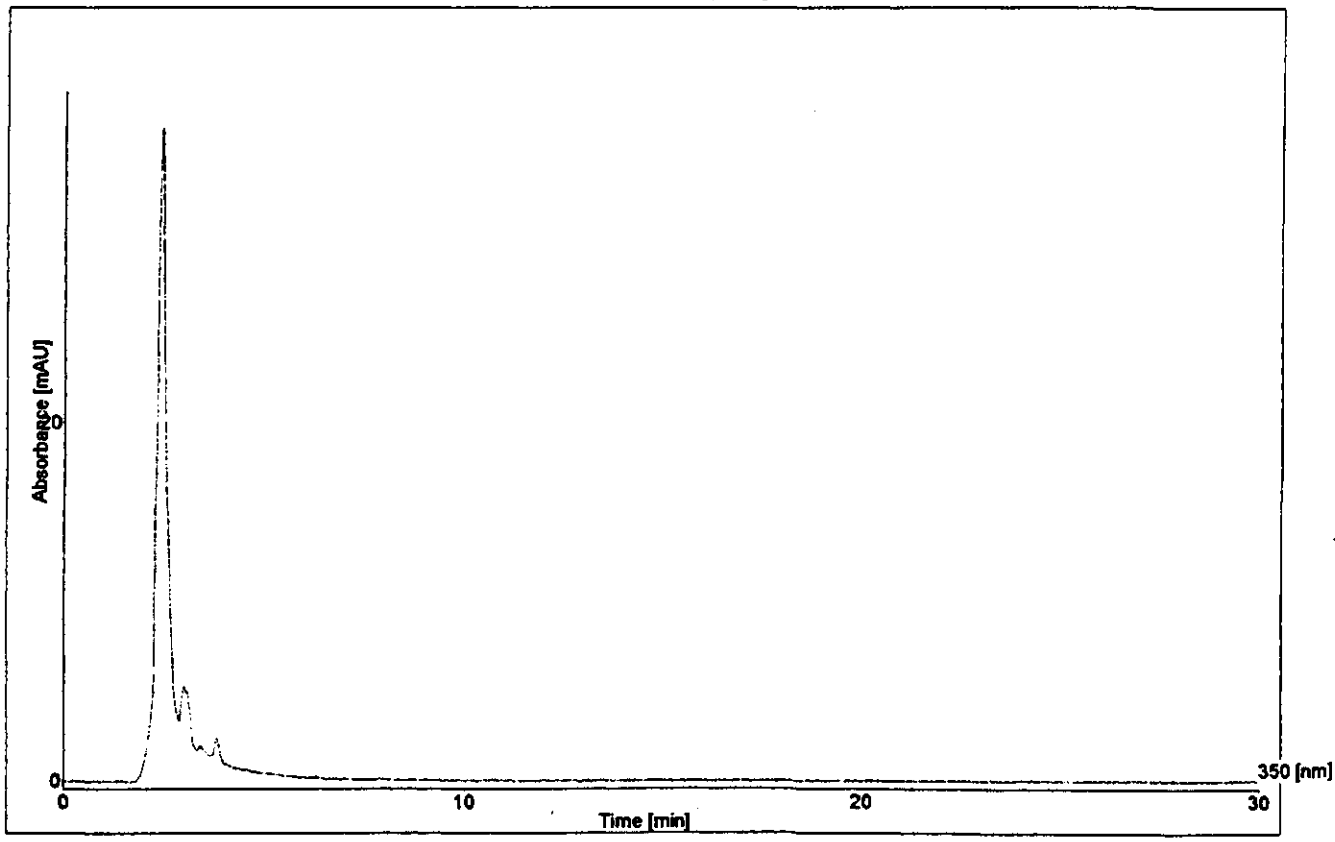
Vial # = 9 Rack # = 0
Injection Date : 2-Dec-2000 1:20:04
Curr. Date : 19-Dec-2000 12:47:40
User : KL
Group : NICARB
Control Method :

#	Name	RT	Height[uAU]	Area[uAU.Sec]	%Area
1	NICARB	6.773	22083	230900.153	100.00

Total Area of Peak = 230900.153 [uAU.Sec]

File Name :D:\BART_DATA\nicarb\NICARB2311023.DA1
Acquisition Date :24 Nov 2000 at 04:28:32 AM +00:00 GMT
Operator Name :
Acquisition Time [min] :30.00
Tacc [Sec] =0.80 Wacc [nm] =4.0
Wavelength Range (250-450 nm)
Autozero [min] :0.00
Info :
a3009164

BLANK FEED



Quantitative Analysis Report

Sample Type :Unknown
Quantitation By :Height

#	Peak Name	Rt	WL	Area	Height	NPlates	Quantity
		[min]	[nm]	[mAU.Sec]	[mAU]		

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 27

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: **Task 4 COLLABORATIVE STUDY**
Lab-name:
Contact person:

e-mail:
fax:
telephone:

Date of analysis:

Analyte: **NICARBAZIN**

	Unit	Result (mg/kg)
Sample code		
274511		20,03
274539		not detectable
274548		42,88
274556		20,05
274558		not detectable
274569		114,67
274618		252,95
274623		119,21
274660		44,46
274691		260,99

Unit / Sample	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture	11698	11458

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Date(s) of analysis: ....16.10. - 20.10.2000.....

Dilution factor of the samples:

- Feed samples (specify for which feed samples): ....f = 2 for 274618 and 274691, else f = 1.....
- Premixture: ....f = 25.....

Chromatographic conditions:

- Column:
  - ☒ As described in the method
  - ☐ Other: ..... but according to the catalogue of the firm Waters the particle size of the reacted silica in Nova-Pak columns is always 4 µm in diameter
- Mobile phase:
  - ☒ As described in the method
  - ☐ Other: .....
- Flow-rate: .....1,0.... ml/min
- Injection volume: ..20.....µl
- Retention time of nicarbazin: ...4,1... min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

Recovery results:

- Percentage recovery: .....99,6 %
- Single / duplicate determinations: ☒ single ☐ duplicate
- If duplicate, please give both percentages: ..... % and ..... %
- Spiking level: ..100... mg/kg

16.10.

Result Table

#	Retention Time (min)	Type	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	Baseline Start (min)	Baseline End (min)	Slope	Offset	% Area
1	4.058	Unknown	45004	3556	MM	3.883	4.617	3.883	4.617	-0.010909	-0.017635	100.00

Result Table

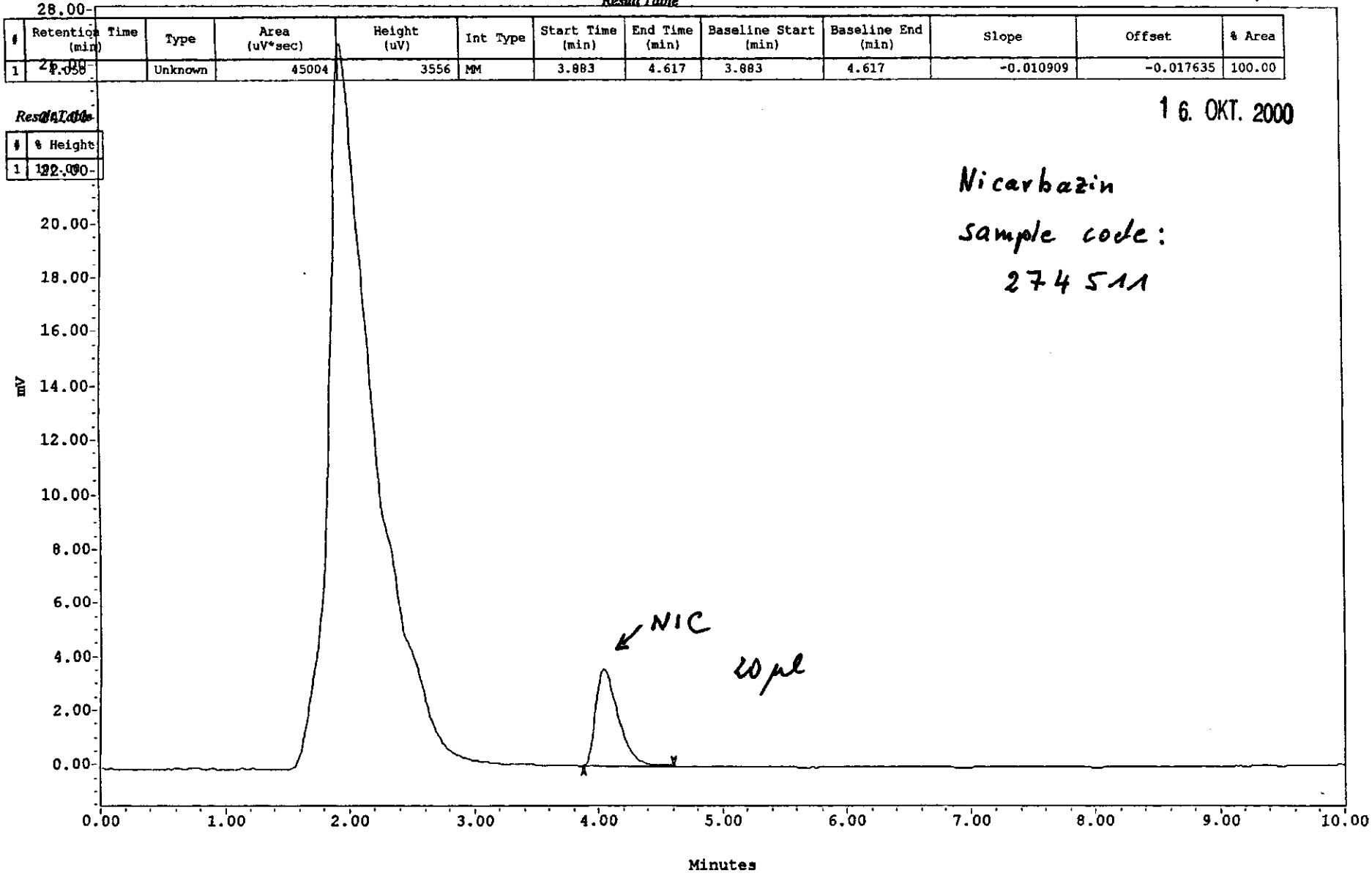
#	% Height
1	100.00

16. OKT. 2000

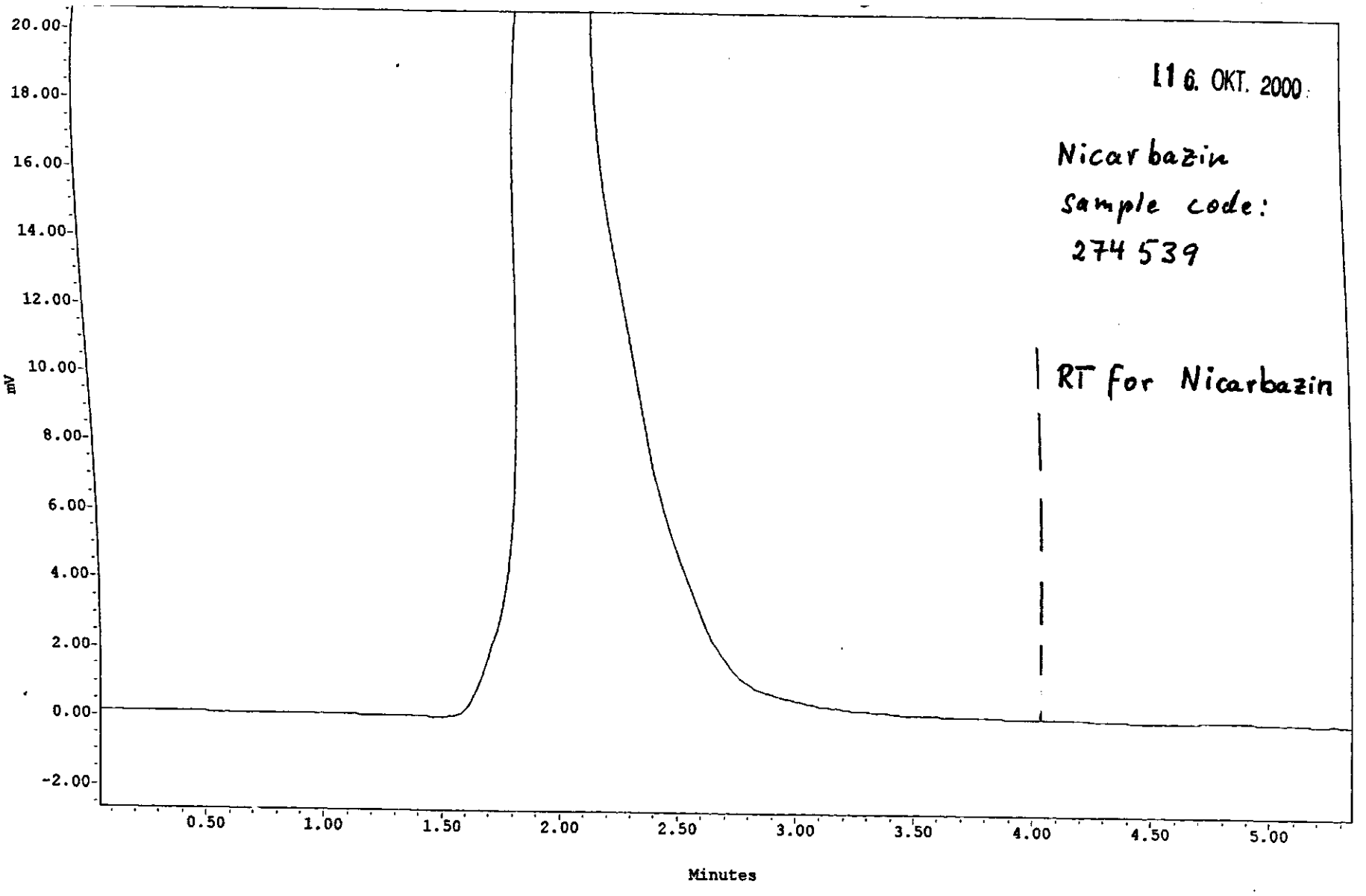
Nicarbazin

sample code:

274511



SampleName: NIC 1 Vial: 11 Inj: 1 Ch: SATIN Type: Unknown Page: 1 of 1



SampleName: NIC 2 Vial: 12 Inj: 1 Ch: SATIN Type: Unknown

16.10.

Result Table

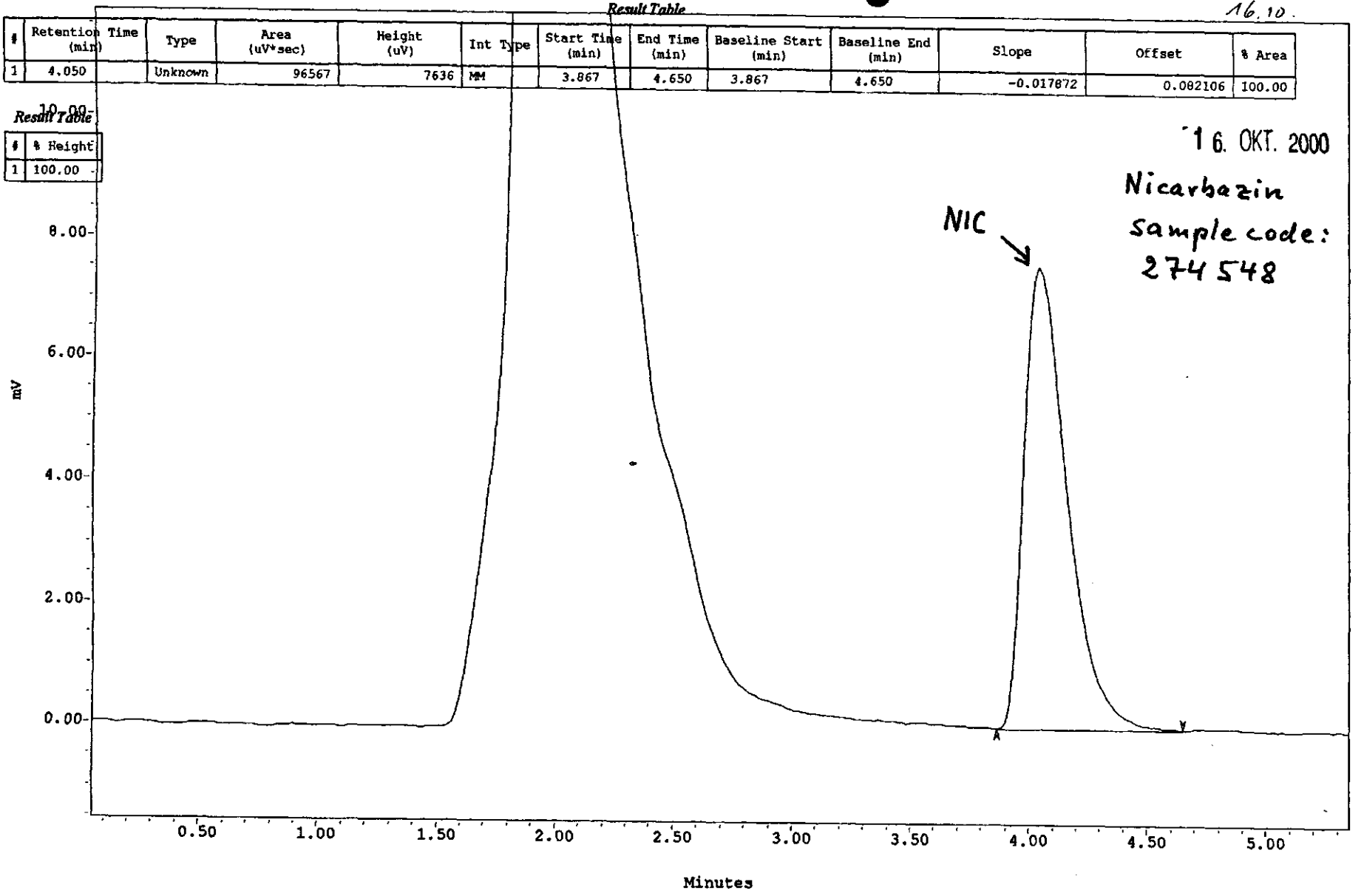
#	Retention Time (min)	Type	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	Baseline Start (min)	Baseline End (min)	Slope	Offset	% Area
1	4.050	Unknown	96567	7636	MM	3.867	4.650	3.867	4.650	-0.017872	0.082106	100.00

Result Table

#	% Height
1	100.00

16. OKT. 2000

Nicarbazin
sample code:
274548



SampleName: NIC 3 Vial: 13 Inj: 1 Ch: SATIN Type: Unknown Page: 1 of 1

17

Result Table

#	Retention Time (min)	Type	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	Baseline Start (min)	Baseline End (min)	Slope	Offset	% Area
1	4.133	Unknown	43687	3340	MM	3.950	4.650	3.950	4.650	-0.057143	0.236714	100.00

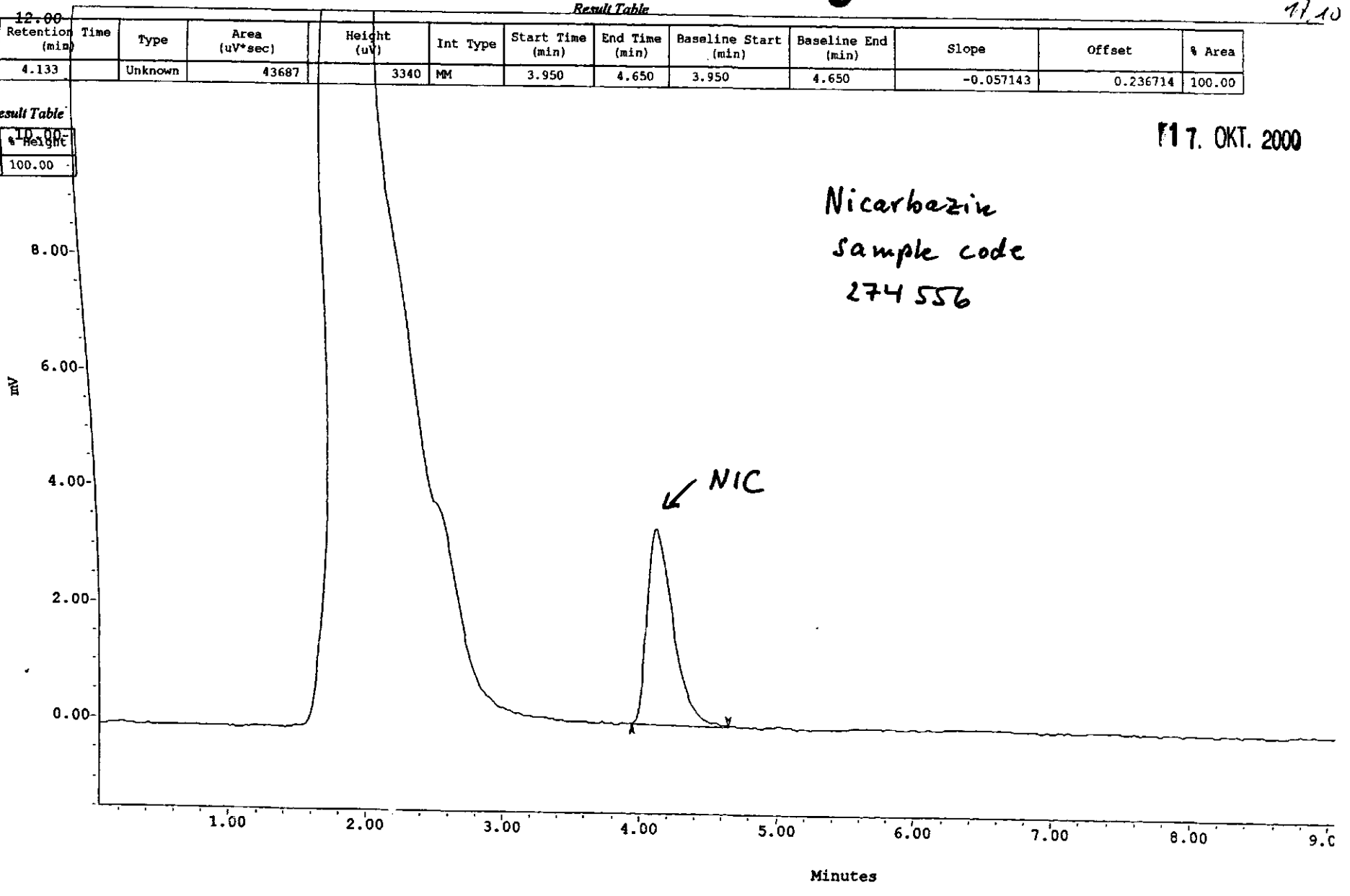
Result Table

#	% Height
1	100.00

17.10

17. OKT. 2000

Nicarbazin
sample code
274556



SampleName: Nic4 Vial: 10 Inj: 1 Ch: SATIN Type: Unknown

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 29

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: **Task 4 COLLABORATIVE STUDY**
Lab-name:
Contact person: e-mail:
fax:
telephone:
Date of analysis:

Analyte: **NICARBAZIN**

	Unit	Result (mg/kg)
Sample code		
294530		21,0
294594		0
294612		248,0
294614		0
294624		126,9
294628		46,1
294629		125,5
294644		23,0
294646		45,6
294650		253,5

Unit / Sample	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture	11220,0	10199,4

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Date(s) of analysis: ..... 23 : 11 : 2000 .....

Dilution factor of the samples:

- Feed samples (specify for which feed samples): .....
  .....
- Premixture: .....

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ☑ Other: .... Nova Pack , 250 x 4,6 mm ; C18 ; 4 µ .....
- Mobile phase:
  - ☑ As described in the method
  - ☐ Other: .....
- Flow-rate: ..... 1,0 ..... ml/min
- Injection volume: .. 20 ... µl
- Retention time of nicarbazin: .45.. min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

Recovery results:

- Percentage recovery: ..... %
- Single / duplicate determinations: ☐ single ☑ duplicate
- If duplicate, please give both percentages: 88.. % and 102,5 %
- Spiking level: ... 100 mg/kg

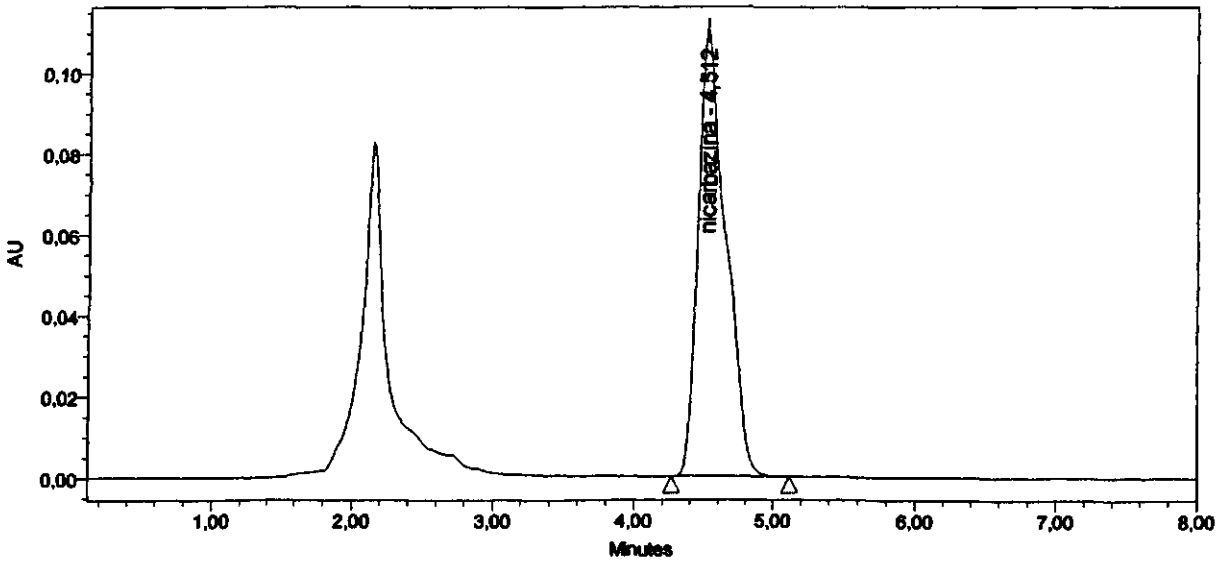
nicarbazina relatório Report

Reported by User: System | Project Name: Nicarbazina

SAMPLE INFORMATION

Sample Name:	nicarbazina 612II	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	27-11-2000 19:42:21
Vial:	9	Acq. Method Set:	instrumental nicarbazina
Injection #:	1	Date Processed:	28-11-2000 11:53:35
Injection Volume:	20,00 ul	Processing Method:	process nicarbazina27_11
Run Time:	8,0 Minutes	Channel Name:	Wln Ch1
Sample Set Name:	nicarbazina sample set	Proc. Chnl. Descr.:	PDA 355,0 nm

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1	nicarbazina	4,512	1536992	111218	16,500	ug/ml

(248 mg/kg)

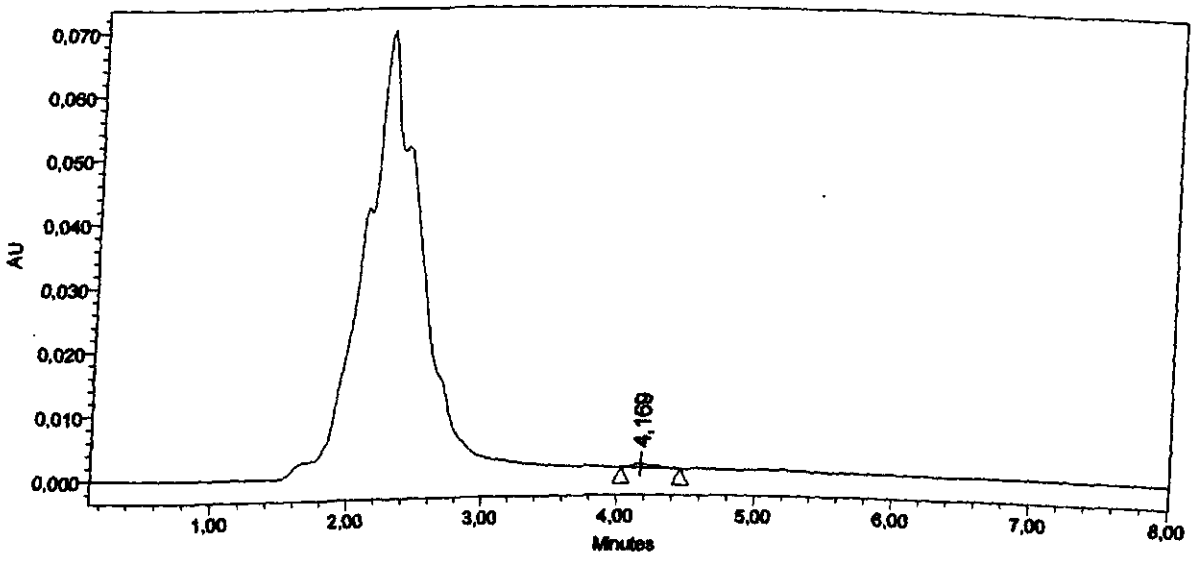
nicarbazina relatório Report

Reported by User: System | Project Name: Nicarbazina

SAMPLE INFORMATION

Sample Name:	nicarbazina 594	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	30-11-2000 13:19:07
Vial:	28	Acq. Method Set:	instrumental nicarbazina
Injection #:	1	Date Processed:	30-11-2000 14:28:31
Injection Volume:	20,00 ul	Processing Method:	process nicarbazina27_11
Run Time:	8,0 Minutes	Channel Name:	WWn Ch1
Sample Set Name:	nicarbazina sample set	Proc. Chnl. Descr.:	PDA 355,0 nm

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1		4,169	5892	578		
2	nicarbazina	4,504			---	

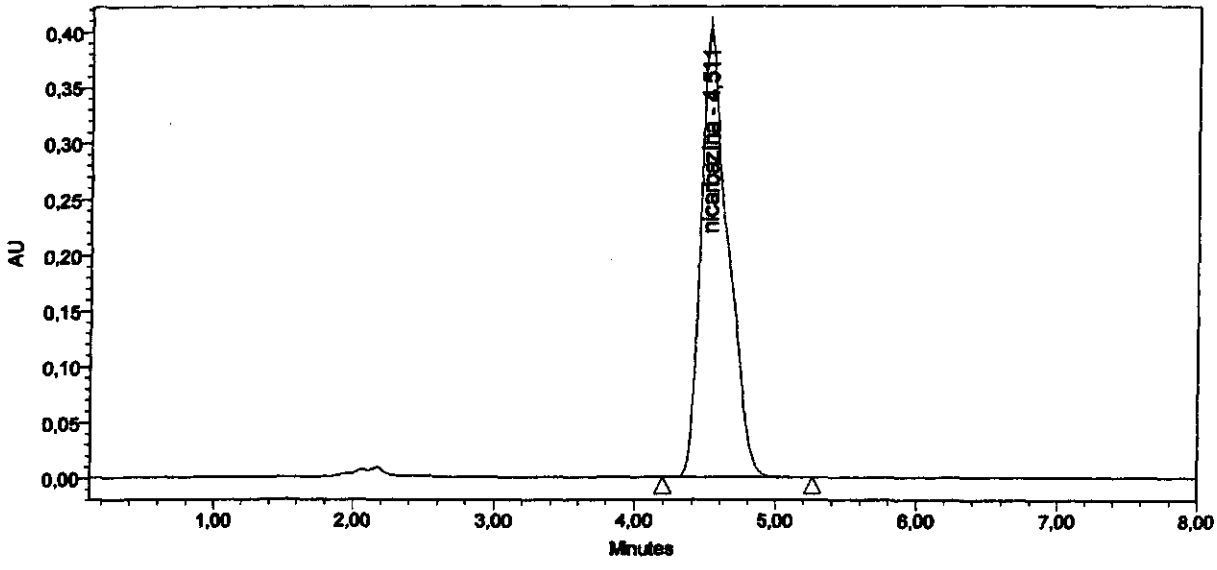
nicarbazina relatório Report

Reported by User: System Project Name: Nicarbazina

SAMPLE INFORMATION

Sample Name:	nicarbazina pré mistura I	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	27-11-2000 22:45:49
Vial:	18	Acq. Method Set:	instrumental nicarbazina
Injection #:	1	Date Processed:	28-11-2000 11:59:44
Injection Volume:	20,00 ul	Processing Method:	process nicarbazina27_11
Run Time:	8,0 Minutes	Channel Name:	Wln Ch1
Sample Set Name:	nicarbazina sample set	Proc. Chnl. Descr.:	PDA 355,0 nm

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1	nicarbazina	4,511	5455895	403032	56,100	ug/ml

(1,1 mg | kg)

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 30

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: **Task 4 COLLABORATIVE STUDY**
Lab-name:
Contact person: e-mail:
fax:
telephone:

Date of analysis:

Analyte: **NICARBAZIN**

Sample code	Unit	Result (mg/kg)
304521		146
304532		24
304562		261
304563		20
304568		38
304584		120
304589		< 3
304626		48
304667		< 3
304682		274

Sample	Unit	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture		11920	12000

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

30

Date(s) of analysis: ....11/12/00.....

Dilution factor of the samples:

- Feed samples (specify for which feed samples): ..samples 304562 and 304682 ..2,5g to 100ml then 5ml to 50ml.....
- Premixture: ..0,5g to 100ml then 2ml to 50ml.....

Chromatographic conditions:

- Column:
  - ☒ As described in the method
  - ☐ Other: .....
- Mobile phase:
  - ☒ As described in the method
  - ☐ Other: .....
- Flow-rate: ..1.0..... ml/min
- Injection volume: 20....µl
- Retention time of nicarbazin: 6.8 min

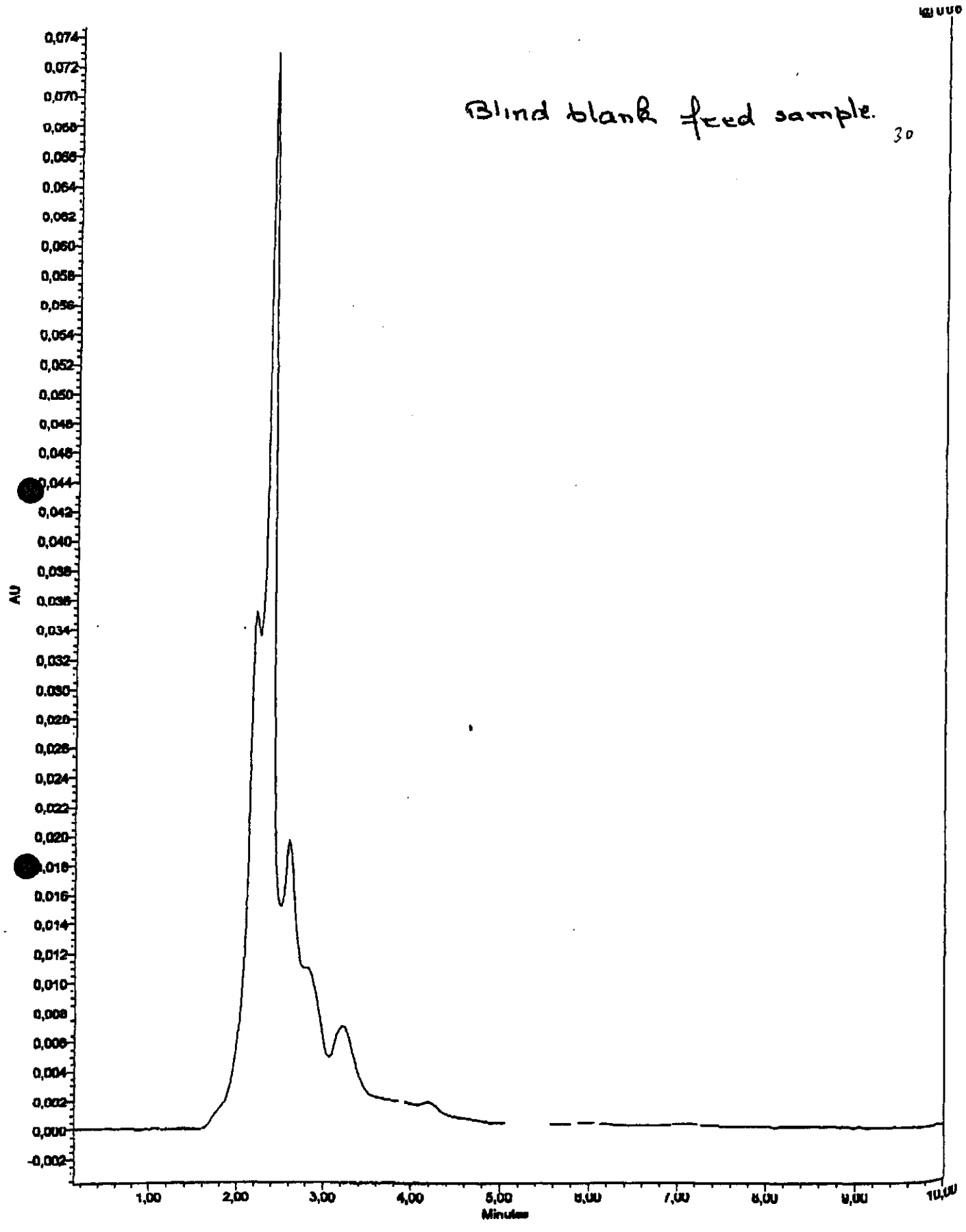
Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

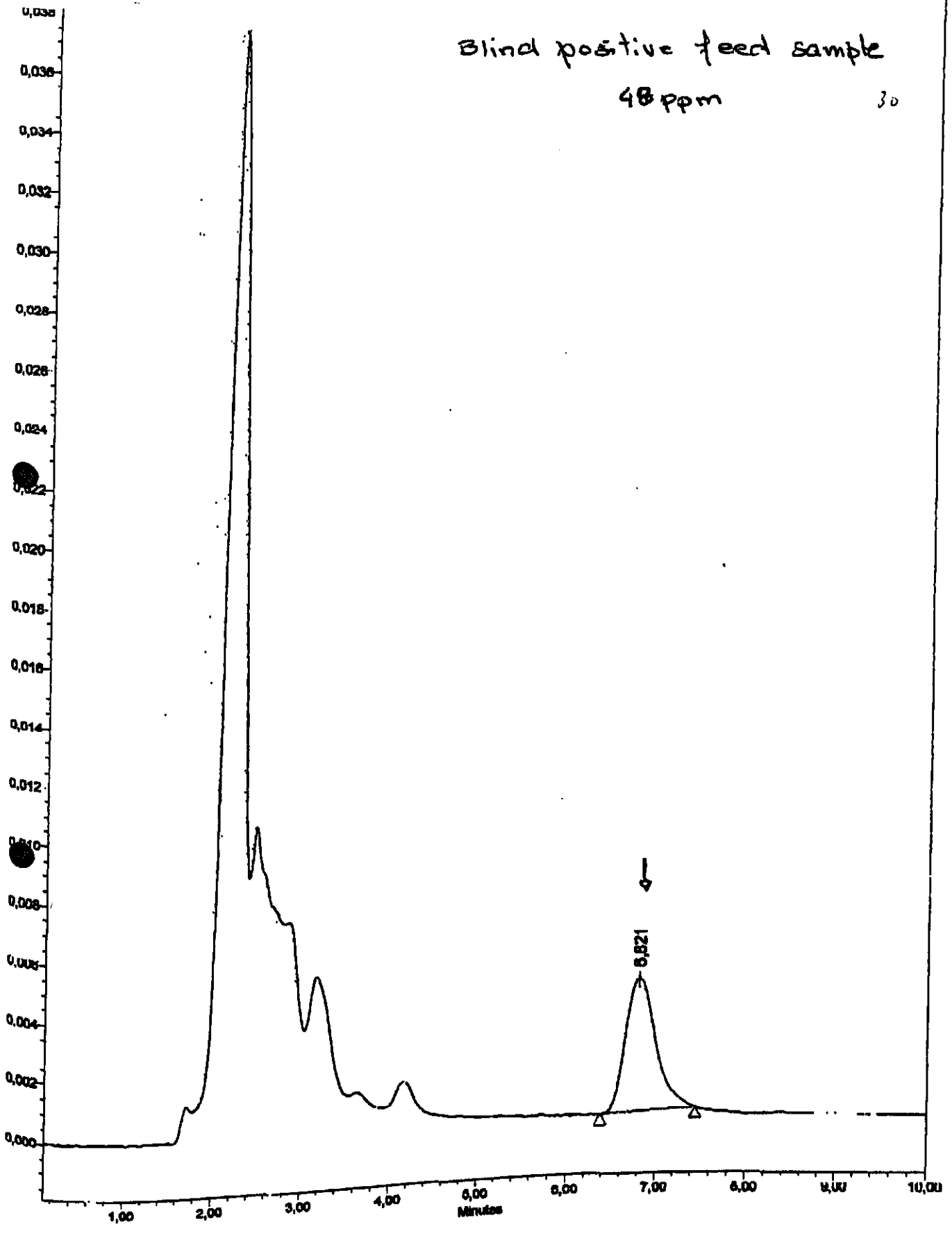
Recovery results:

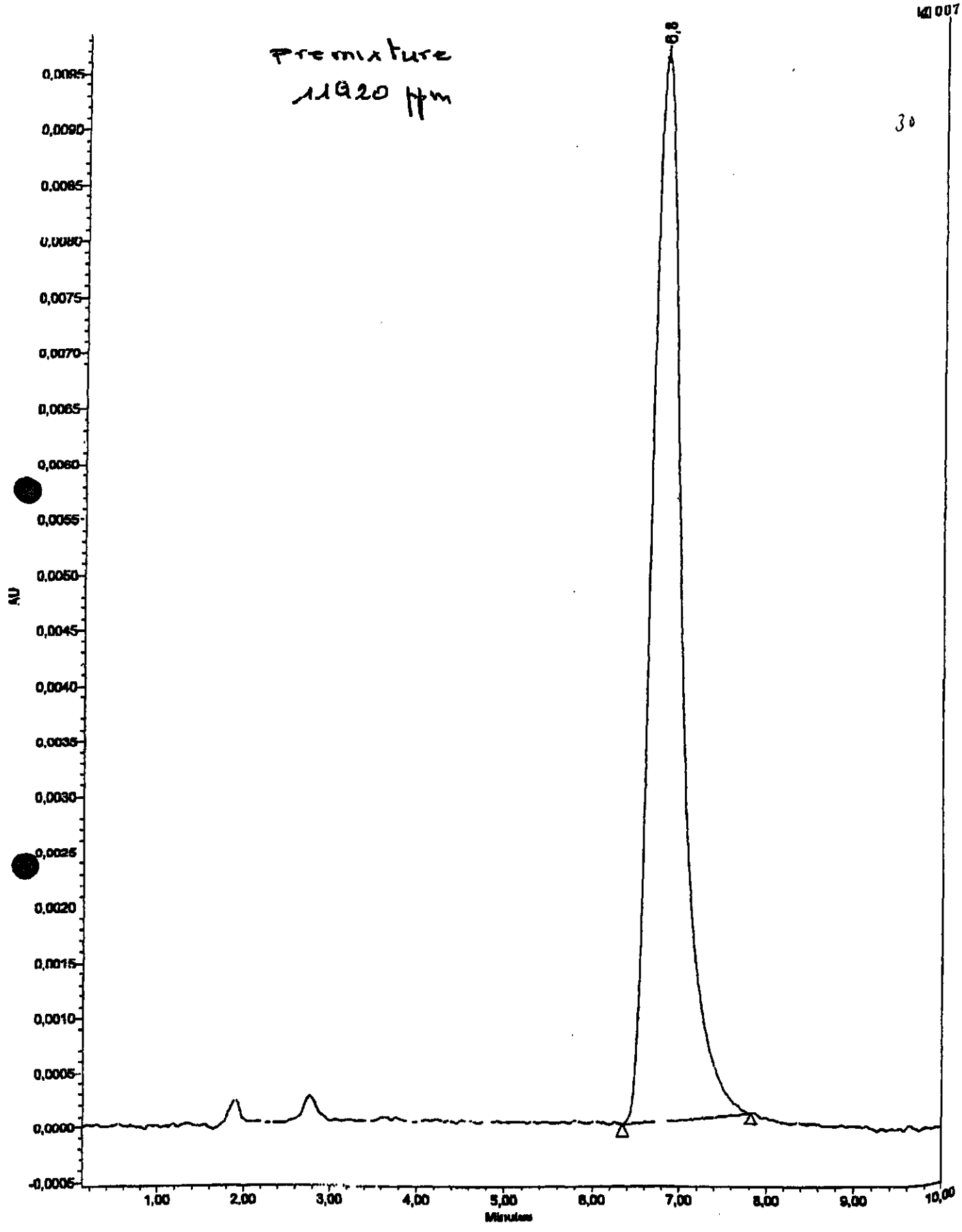
- Percentage recovery: 99.3%
- Single / duplicate determinations: ☒ single ☐ duplicate
- If duplicate, please give both percentages: ..... % and ..... %
- Spiking level: 100.. mg/kg


Blind blank feed sample.
30
AU
Minutes

Blind positive feed sample

48 ppm 30




premixture
11920 Hm
30
007
6,8
AU
0,0095
0,0090
0,0085
0,0080
0,0075
0,0070
0,0065
0,0060
0,0055
0,0050
0,0045
0,0040
0,0035
0,0030
0,0025
0,0020
0,0015
0,0010
0,0005
0,0000
-0,0005
1,00
2,00
3,00
4,00
5,00
6,00
7,00
8,00
9,00
10,00
Minutes

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 31

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: Task 4 COLLABORATIVE STUDY
Lab-name:
Contact person:
e-mail:
fax:
telephone:
Date of analysis:

Analyte: NICARBAZIN

Sample code	Unit	Result (mg/kg)
314570		117
314590		20,6
314595		49,4
314599		266
314609		1,4
314613		45,5
314635		23,1
314636		270
314661		1,5
314680		115

Sample / Unit	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture	10200	11010

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Laboratory: .....

Contact person: .....

Date(s) of analysis: .....25-10-2000.....

Dilution factor of the samples:

- Feed samples (specify for which feed samples): .....undiluted.....
  .....
- Premixture: ..undiluted...range...of...calibration...was...adjusted...to...concentration

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ☒ Other: ...Brownlee...C18...250 mm x 4,6 mm ID.....
- Mobile phase:
  - ☒ As described in the method
  - ☐ Other: .....
- Flow-rate: .....1.... ml/min
- Injection volume: ....20...µl
- Retention time of nicarbazin: ..5,1.. min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

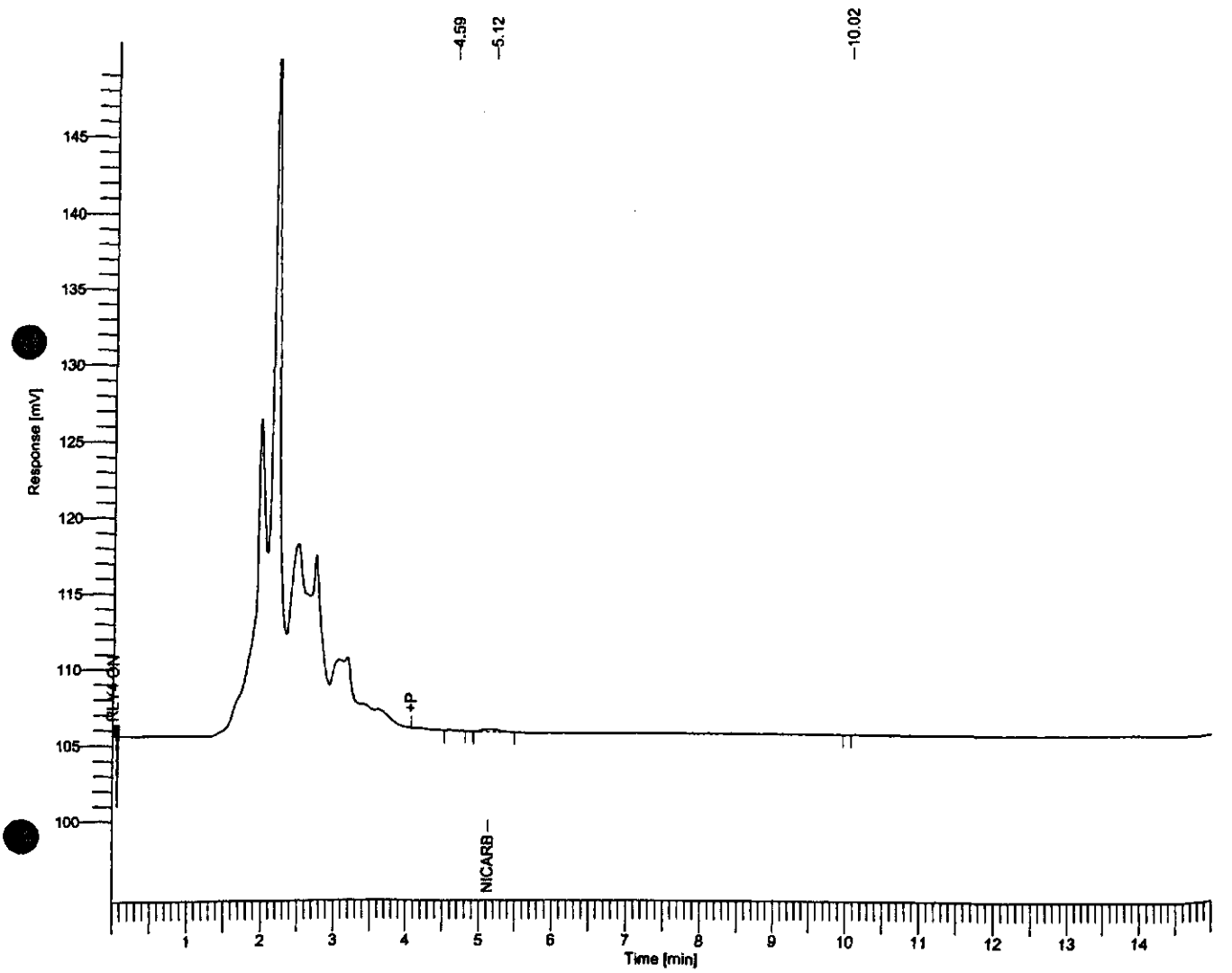
Recovery results:

- Percentage recovery: .99,5. %
- Single / duplicate determinations: ☐ single ☒ duplicate
- If duplicate, please give both percentages: 103,5 % and 95,6 %
- Spiking level: ...100.... mg/kg

Software Version	: 6.1.1.0.0:K20	Date	: 10/26/00 6:57:36 PM
Sample Name	: 00-21052	Data Acquisition Time	: 10/26/00 6:42:28 PM
Instrument Name	: HPLC-3	Channel	: A
Rack/Vial	: 0/0	Operator	:
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 12		

31

Result File : \\rik004s\TCdata\kb residue\HPLC-3\nic canfas 251000-012.rst
Sequence File : \\rik004s\TCdata\KB Residue\HPLC-3\nicarbazin canfas 251000.seq



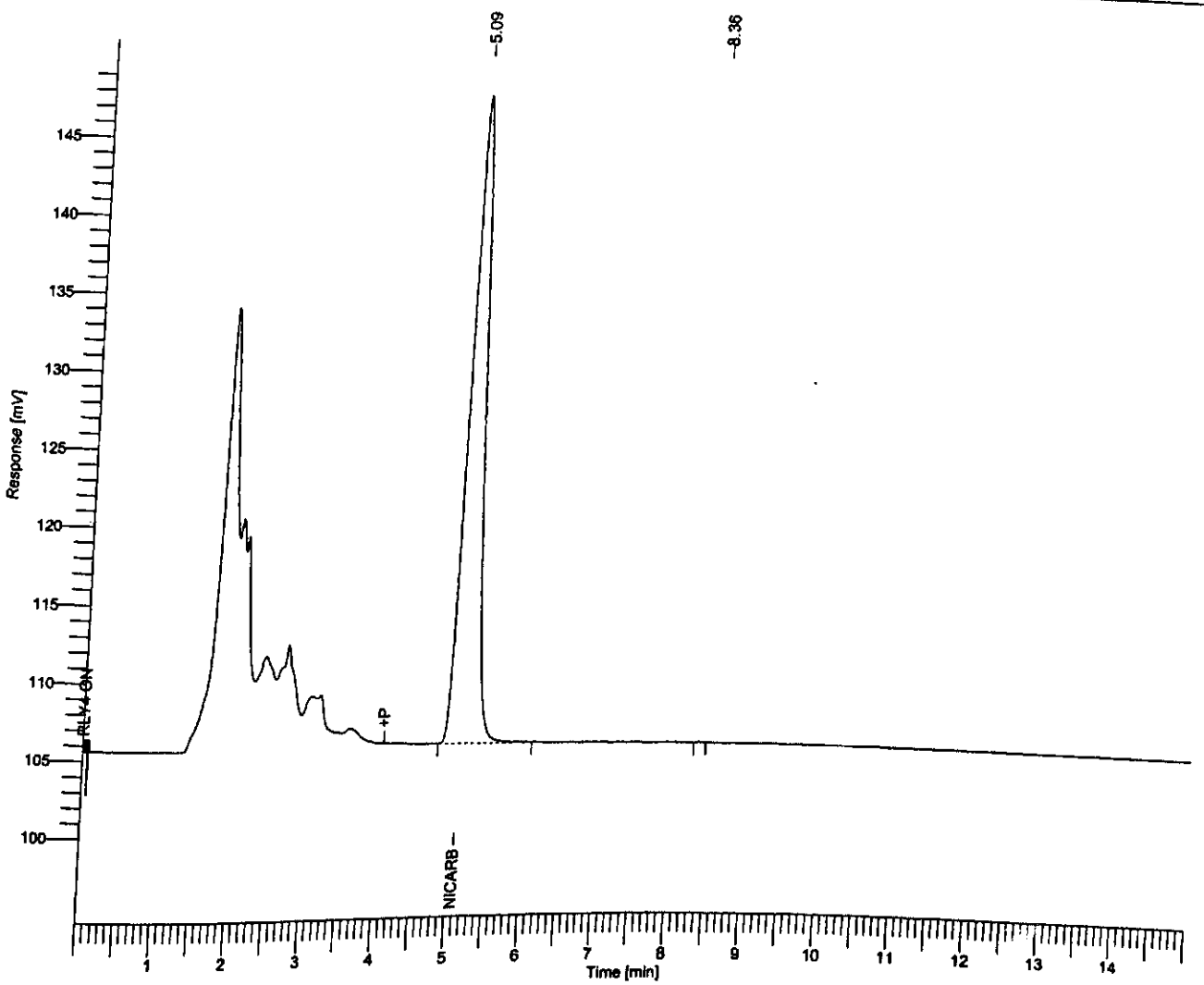
Nicarbazin CANFAS ringtest

Peak #	Component Name	Time [min]	Area [µV·s]	Height [µV]
1		4.59	497	50
2	Nicarbazin	5.12	3193	193
3		10.02	101	25
			3791	269

314609

Software Version	: 6.1.1.0.0:K20	Date	: 10/26/00 6:40:40 PM
Sample Name	: 00-21051	Data Acquisition Time	: 10/26/00 6:25:33 PM
Instrument Name	: HPLC-3	Channel	: A
Rack/Vial	: 0/0	Operator	:
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 11		

Result File : \\rik004s\TCdata\kb residue\HPLC-3\nic canfas 251000-011.rst
Sequence File : \\rik004s\TCdata\KB Residue\HPLC-3\nicarbazin canfas 251000.seq



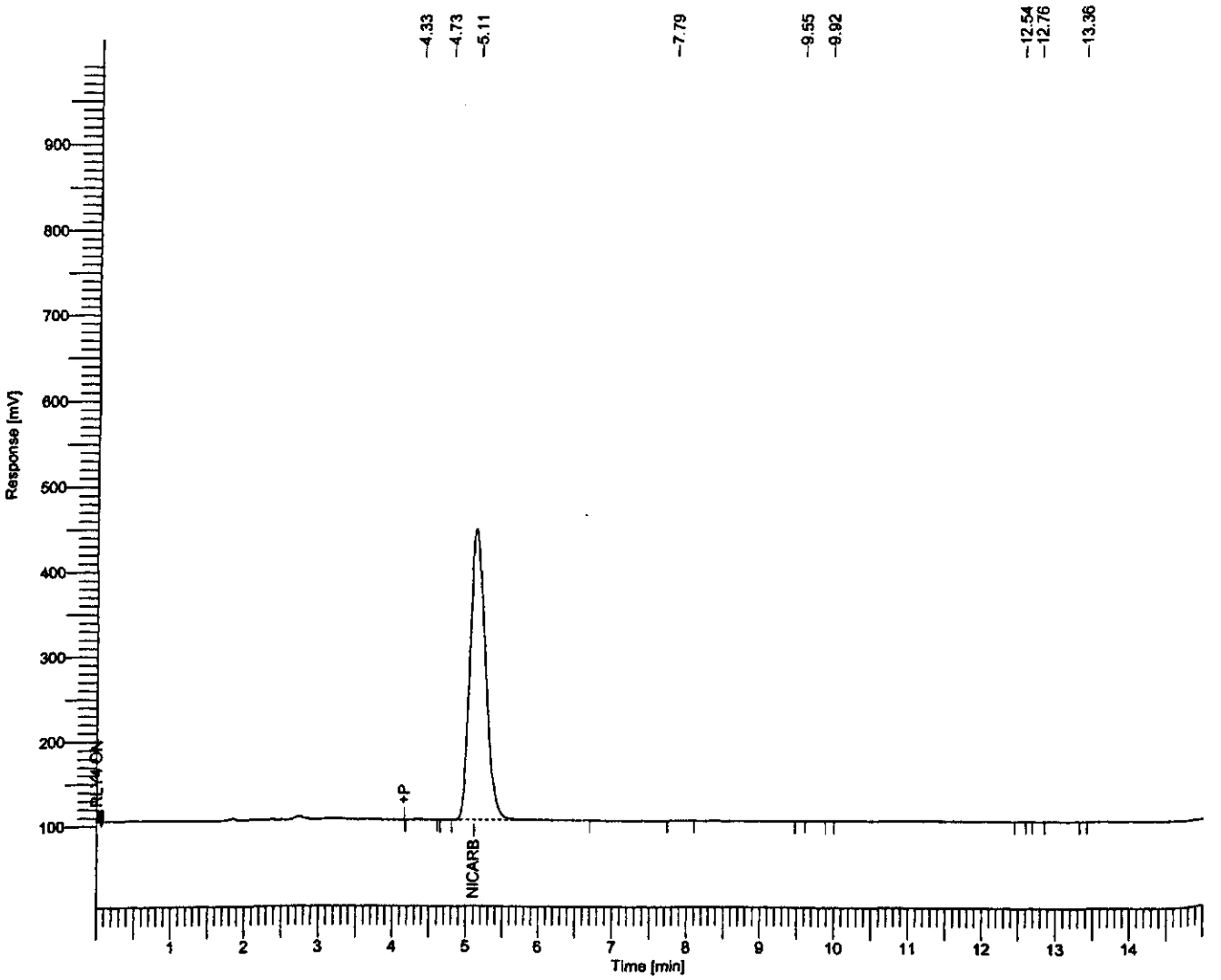
Nicarbazin CANFAS ringtest

Peak #	Component Name	Time [min]	Area [µV·s]	Height [µV]
1	Nicarbazin	5.09	608643	41755
2		8.36	131	29
			608774	41784

Software Version	: 6.1.1.0.0:K20	Date	: 10/27/00 1:43:40 AM
Sample Name	: 00-21058-1	Data Acquisition Time	: 10/27/00 1:28:32 AM
Instrument Name	: HPLC-3	Channel	: A
Rack/Vial	: 0/0	Operator	:
Sample Amount	: 1.000000	Dilution Factor	: 1.000000
Cycle	: 36		

3/

Result File : \\rik004s\TCdata\kb residue\HPLC-3\nic canfas 251000-036.rst
Sequence File : \\rik004s\TCdata\KB Residue\HPLC-3\nicarbazin canfas 251000.seq



Nicarbazin CANFAS ringtest

Peak #	Component Name	Time [min]	Area [µV·s]	Height [µV]
1		4.33	5410	453
2		4.73	347	52
3	Nicarbazin	5.11	5030613	341127
4		7.79	332	23
5		9.55	91	24
6		9.92	46	12
7		12.54	135	25
8		12.76	83	21
9		13.36	74	18
			5037134	341756

Premix

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 32

First results, CANFAS method was not followed (see paragraph 5.1)

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle:
Lab-name:
Contact person:

e-mail:
fax:
telephone:

Date of analysis:

Analyte: **NICARBAZIN**

Unit / Sample code	Result 1 (mg/kg)	Result 2 (mg/kg)
324506	180,29	179,84
324507	87,97	82,74
324517	32,75	33,78
324561	34,13	33,35
324576	182,18	177,74
324591	Negative	Negative
324593	14,30	13,45
324625	80,10	83,26
324640	16,36	12,16
324675	Negative	Negative

Unit / Sample	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture	7642,00	7642,50

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Date(s) of analysis: 2000.11.23

Dilution factor of the samples:

- Feed samples (specify for which feed samples): 324507, 324517, 324561, 324593, 324625, 324640 and 324675 – Dil. Factor 100

324506, 324576 and 324591 – Dil. Factor 200

- Premixture: Dil. Factor 4000

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ☒ Other: Waters Symmetry, C18, 5 um, 4.6mmX250mm (Part N° WAT 054215)
- Mobile phase:
  - ☒ As described in the method
  - ☐ Other: .....
- Flow-rate: 1.4 ml/min
- Injection volume: 20 (µL)
- Retention time of nicarbazin: 4.61 min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

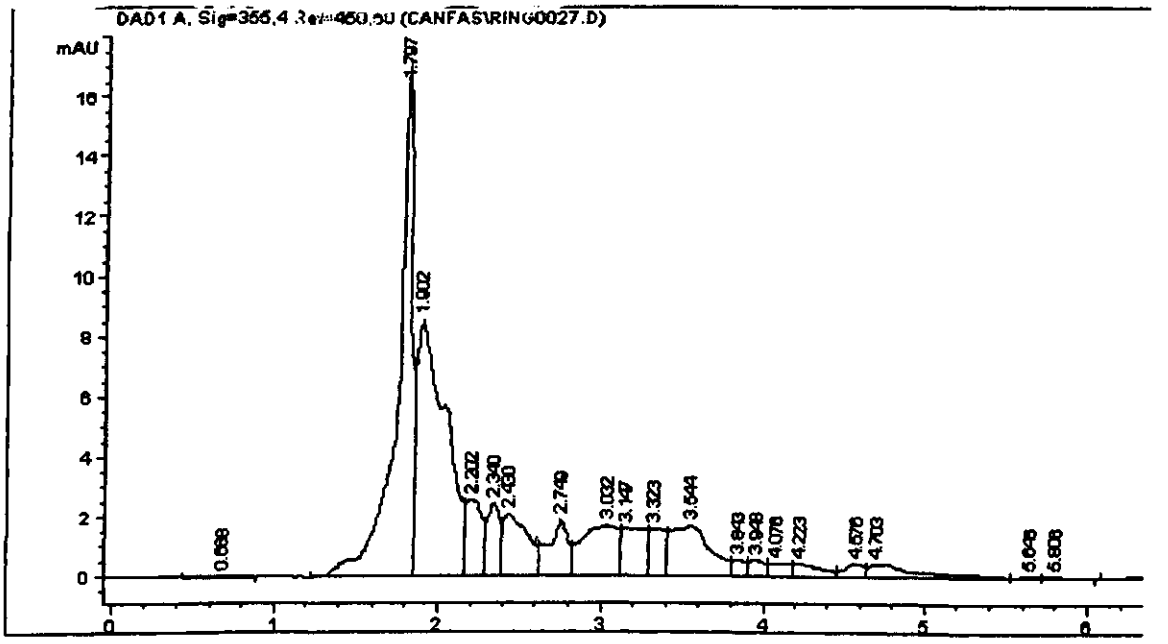
Recovery results:

- Percentage recovery: 98.3 %
- Single/duplicate determinations: ☐ single ☒ duplicate
- If duplicate, please give both percentages: 100.25% and 96.26%
- Spiking level: 100 mg/kg

Data File C:\HPC

code 324591,

Injection Date	: 11/24/00 3:19:39 AM	Seq. Line	: 27
Sample Name	: 324591//	Vial	: 27
Acq. Operator	:	Inj	: 1
		Inj Volume	: 20 µl
Acq. Method	: C:\HPCHEM\1\METHODS\NICARBAZ.M		
Last changed	: 11/23/00 11:41:15 PM		
Analysis Method	: C:\HPCHEM\1\METHODS\LAV2.M		
Last changed	: 11/24/00 12:03:20 PM (modified after loading)		



External Standard Report

Sorted By	:	Signal
Calib. Data Modified	:	Friday, November 24, 2000 11:53:03 AM
Multiplier	:	1.0000
Dilution	:	1.0000

Signal 1: DAD1 A, Sig=355,4 Ref=450,50

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/ml]	Grp	Name
4.576	VV	3.99061	0.00000	0.00000		Nicarbazin

Totals : 0.00000

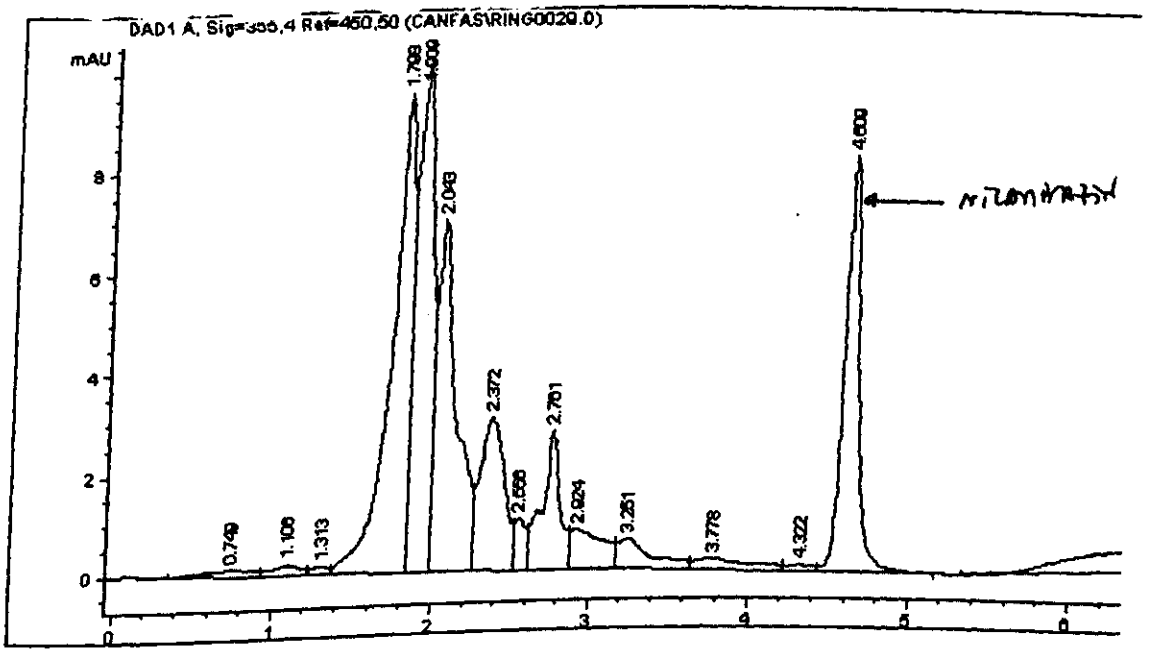
Results obtained with enhanced integrator!
1 Warnings or Errors :

Data File C:\HPCHEM\1\DATA\CANFAS\RING0029.D Sample Name: 324593/

code 324593,

Injection Date : 11/24/00 3:37:35 AM Seq. Line : 29
Sample Name : 324593/ Vial : 29
Acq. Operator : Inj : 1
Inj Volume : 20 µl

Acq. Method : C:\HPCHEM\1\METHODS\NICARBAZ.M
Last changed : 11/23/00 11:41:15 PM
Analysis Method : C:\HPCHEM\1\METHODS\LAV2.M
Last changed : 11/24/00 12:03:57 PM
(modified after loading)



External Standard Report

Sorted By : Signal
Calib. Data Modified : Friday, November 24, 2000 11:53:03 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=355,4 Ref=450,50

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/ml]	Grp	Name
4.609	VB	54.48381	6.56050e-3	3.57441e-1		Nicarbazin
Totals :				3.57441e-1		

Results obtained with enhanced integrator!

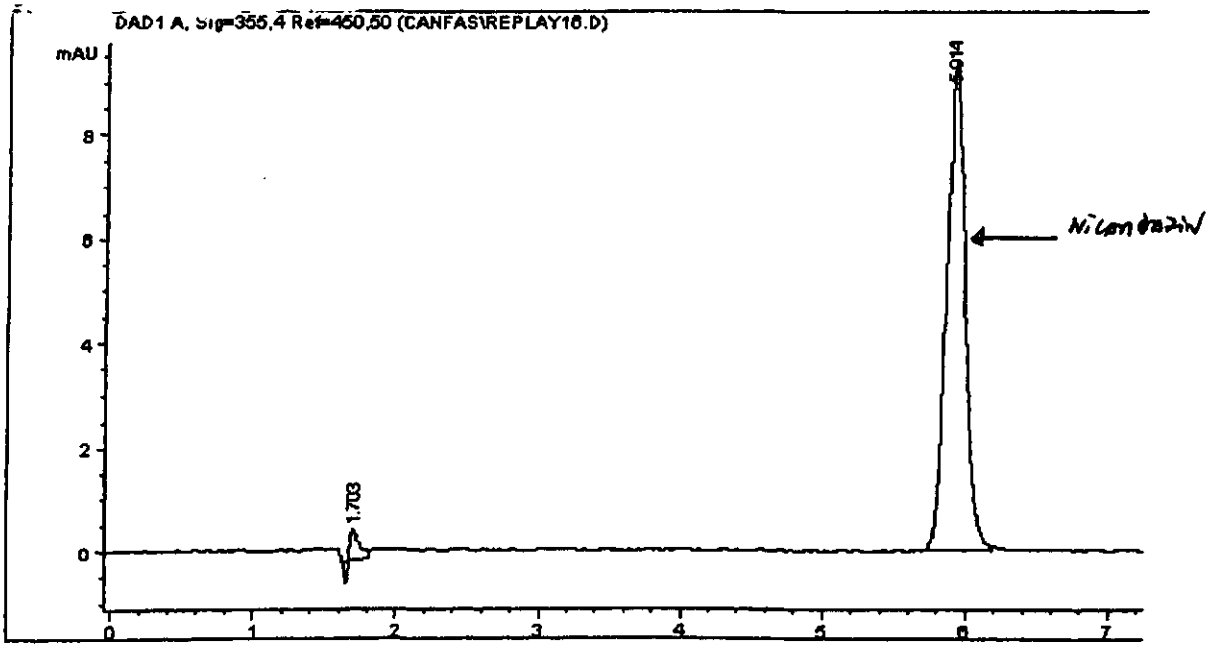
32

Data File C:\HPCHEM\1\DATA\CANFAS\REPLAY16.D Sample Name: PM

code Premix,_

PM Liver

Injection Date	: 11/29/00 9:45:56 PM	Seq. Line	: 16
Sample Name	: PM	Vial	: 16
Acq. Operator	:	Inj	: 1
		Inj Volume	: 20 µl
Acq. Method	: C:\HPCHEM\1\METHODS\NICARBAZ.M		
Last changed	: 11/29/00 7:34:13 PM		
Analysis Method	: C:\HPCHEM\1\METHODS\NICARBAZ.M		
Last changed	: 11/30/00 10:26:05 AM (modified after loading)		



External Standard Report

Sorted By	:	Signal
Calib. Data Modified	:	Thursday, November 30, 2000 10:08:18 AM
Multiplier	:	1.0000
Dilution	:	1.0000

Signal 1: DAD1 A, Sig=355,4 Ref=450,50

RetTime [min]	Type	Height [mAU]	Amt/Height	Amount [ug/ml]	Grp	Name
5.914	BB	9.26688	1.03134e-1	9.55728e-1		Nicarbazin

Totals : 9.55728e-1

Results obtained with enhanced integrator!

Instrument 1 11/30/00 10:26:07 AM

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 32

New results, CANFAS method was followed

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: Task 4 COLLABORATIVE STUDY

Lab-name:

Contact person: e-mail:
fax:
telephone:

Date of analysis: 2000.04.12

Analyte: NICARBAZIN

Unit / Sample code	Result 1 (mg/kg)	Result 2 (mg/kg)
324506	266.01	271.08
324507	123.95	112.91
324517	43.88	53.37
324561	47.55	47.73
324576	264.45	262.56
324591	Negative	Negative
324593	23.20	20.85
324625	121.76	128.99
324640	21.22	24.37
324675	Negative	Negative

Unit / Sample	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture	9932.04	10414.06

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Laboratory:

Contact person:

Date(s) of analysis: 2000.04.12

Dilution factor of the samples:

- Feed samples (specify for which feed samples): 324507, 324517, 324561, 324593, 324625, 324640 and 324675 – Dil. Factor 100

324506, 324576 and 324591 – Dil. Factor 200

- Premixture: Dil. Factor 4000

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ☒ Other: Waters Symmetry, C18, 5 um, 4.6mmX250mm (Part N° WAT 054215)
- Mobile phase:
  - ☐ As described in the method
  - ☒ Other: Methanol/Water 65/35 (v/v)
- Flow-rate: 1.4 ml/min
- Injection volume: 20 (µL)
- Retention time of nicarbazin: 8.62 min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

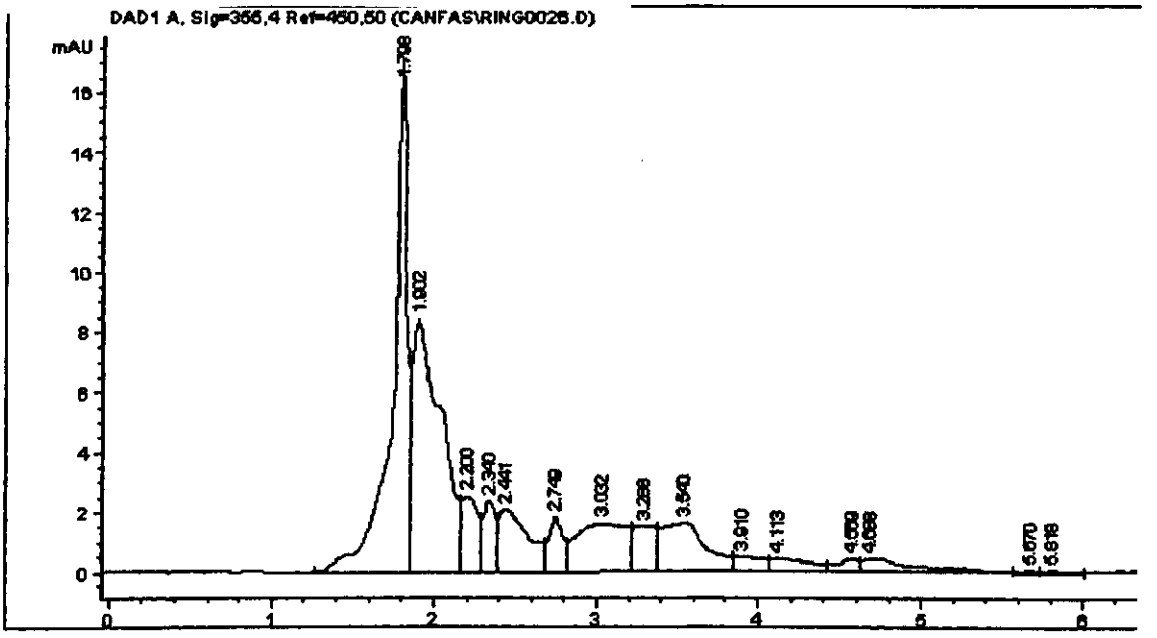
Recovery results:

- Percentage recovery: 100.2 %
- Single/duplicate determinations: ☐ single ☒ duplicate
- If duplicate, please give both percentages: 100.25% and 100.10%
- Spiking level: 100 mg/kg

Data File C:\HPCHEM\1\DATA\CANFAS\RING0026.D Sample Name: 324591/

code 324591, ve

Injection Date : 11/24/00 3:10:38 AM Seq. Line : 26
Sample Name : 324591/ Vial : 26
Acq. Operator : Inj : 1
Inj Volume : 20 µl
Acq. Method : C:\HPCHEM\1\METHODS\NICARBAZ.M
Last changed : 11/23/00 11:41:15 PM
Analysis Method : C:\HPCHEM\1\METHODS\LAV2.M
Last changed : 11/24/00 12:02:57 PM
(modified after loading)



External Standard Report

Sorted By : Signal
Calib. Data Modified : Friday, November 24, 2000 11:53:03 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=355,4 Ref=450,50

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/ml]	Grp	Name
4.569	VV	4.48992	0.00000	0.00000		Nicarbazin

Totals : 0.00000

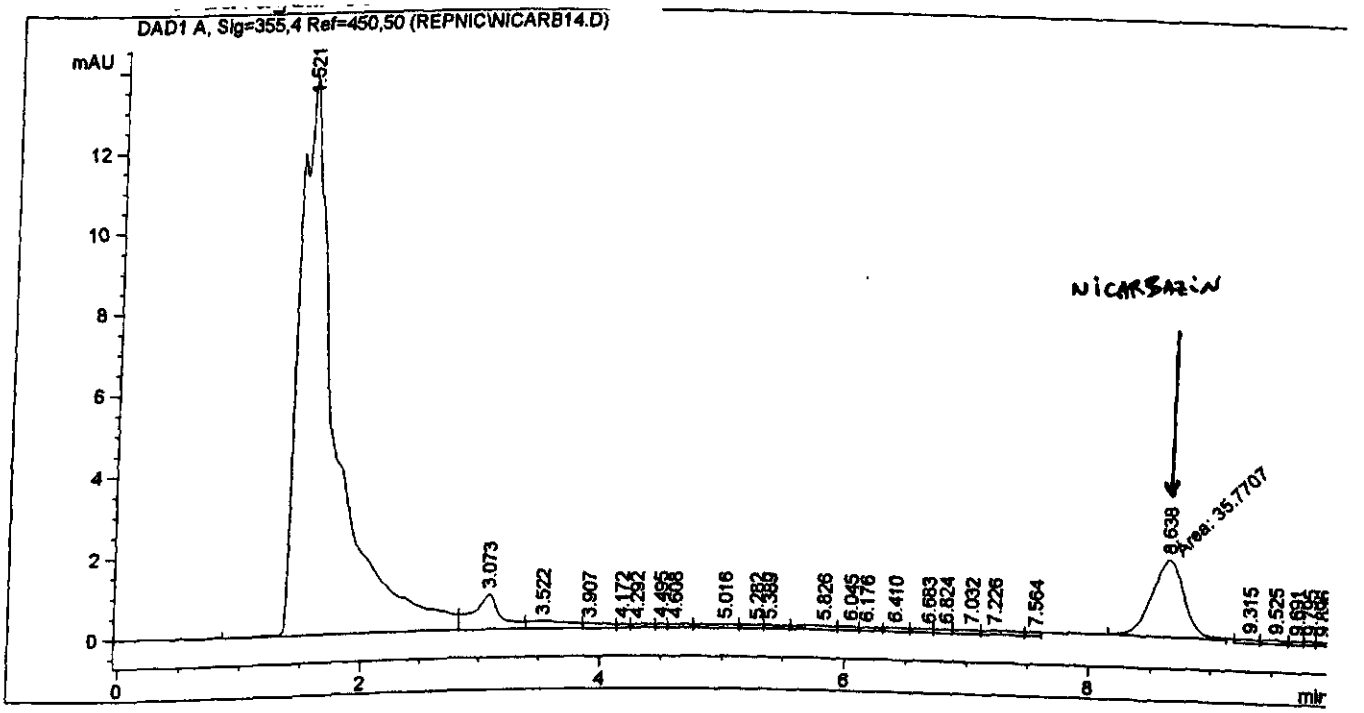
Results obtained with enhanced integrator!
1 Warnings or Errors :

Instrument 1 11/24/00 12:02:58 PM

Data File C:\HPCHEM\1\DATA\REPNIC\NICARB14.D Sample Name: 32459

Sample code 324593 - 2.5004g

Injection Date	: 4/11/01 9:19:30 PM	Seq. Line	: 14
Sample Name	: 324593	Vial	: 11
Acq. Operator		Inj	: 1
		Inj Volume	: 20 µl
Acq. Method	: C:\HPCHEM\1\METHODS\NICARBAZ.M		
Last changed	: 4/11/01 6:40:43 PM		
Analysis Method	: C:\HPCHEM\1\METHODS\LAV2.M		
Last changed	: 4/12/01 10:19:23 AM (modified after loading)		



External Standard Report

Sorted By	:	Signal
Calib. Data Modified	:	4/12/01 10:19:20 AM
Multiplier	:	1.0000
Dilution	:	1.0000

Signal 1: DAD1 A, Sig=355,4 Ref=450,50

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng/ul]	Grp	Name
8.638	MM	35.77074	1.62200e-2	5.80200e-1		Nicarbazin
Totals :				5.80200e-1		

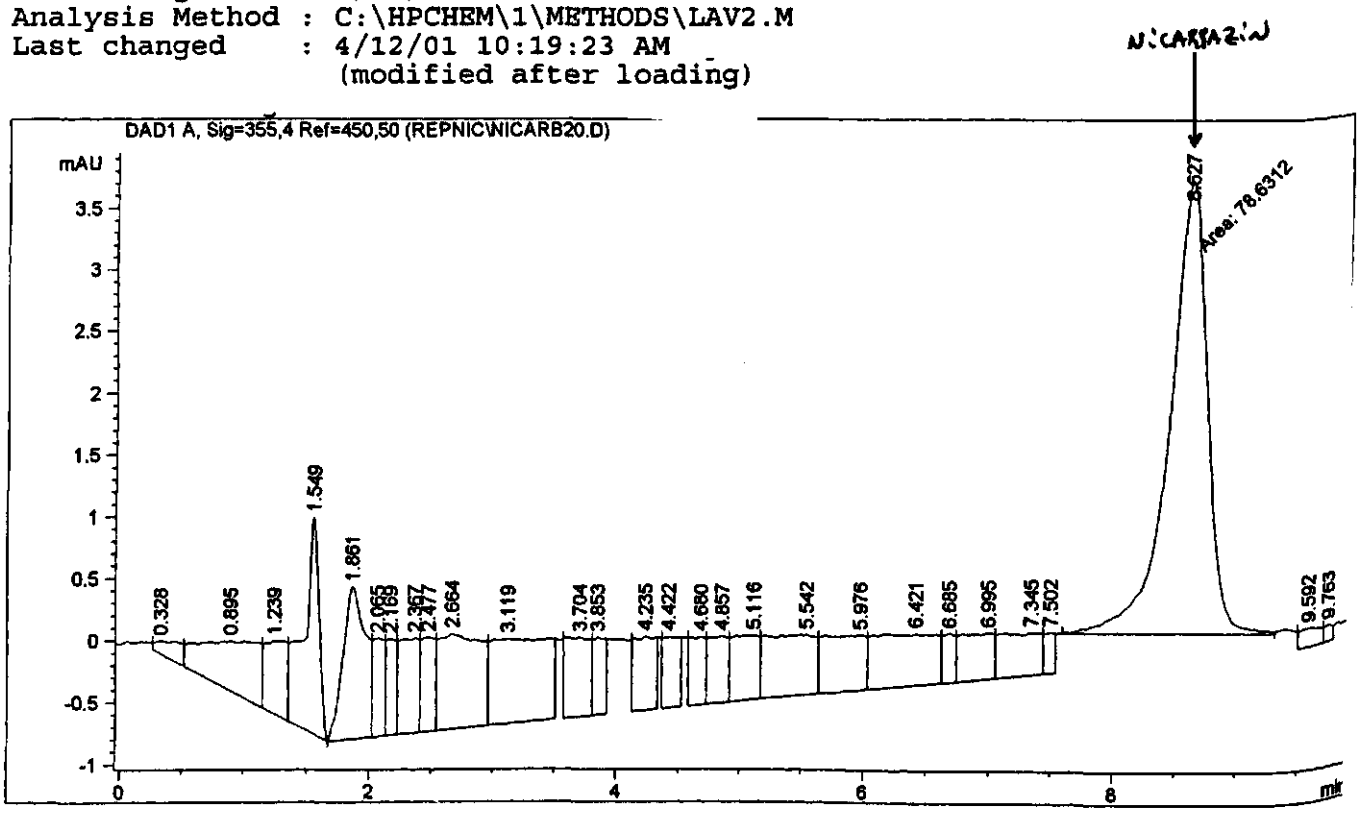
Results obtained with enhanced integrator!

*** End of Report ***

Sample code Pre-mix1 - 0.5009g

```
==========================================================================
Injection Date   : 4/11/01 10:31:49 PM          Seq. Line :  20
Sample Name      : PM1                            Vial :  17
Acq. Operator    :                                 Inj :   1
                                                Inj Volume : 20 µl

Acq. Method      : C:\HPCHEM\1\METHODS\NICARBAZ.M
Last changed     : 4/11/01 6:40:43 PM
Analysis Method  : C:\HPCHEM\1\METHODS\LAV2.M
Last changed     : 4/12/01 10:19:23 AM
                   (modified after loading)
```




```
==========================================================================
                        External Standard Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      4/12/01 10:19:20 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=355,4 Ref=450,50

RetTime  Type      Area       Amt/Area     Amount    Grp   Name
 [min]           [mAU*s]                  [ng/ul]
-------|------|----------|----------|----------|--|-----------------
  8.627 MM       78.63120 1.58174e-2    1.24374     Nicarbazin

Totals :                                 1.24374

 Results obtained with enhanced integrator!
==========================================================================
                          *** End of Report ***
```

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 33

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: **Task 4 COLLABORATIVE STUDY**

Lab-name:
Contact person:
e-mail:
fax:
telephone:

Date of analysis:

Analyte: **NICARBAZIN**

	Unit	Result (mg/kg)
Sample code		
334541		235,8
334565		22,2
334567		107,6
334572		< 2
334573		113,8
334605		46,3
334608		20,4
334615		232,2
334638		< 2
334670		42

Unit / Sample	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture	10260	10550

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Date(s) of analysis: ....13/10/00.....

Dilution factor of the samples:

- Feed samples (specify for which feed samples): .....2g/100 ml.....
  .....
- Premixture: ....2g/100 1/20.....

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ☒ Other: ....C18 ODS.....
- Mobile phase:
  - ☐ As described in the method
  - ☒ Other: ..... 55/45 ammoniumacetaatbuffer 4,8/acetonitrile
- Flow-rate: .....0,6.... ml/min
- Injection volume: 2.0µl
- Retention time of nicarbazin: ..8... min

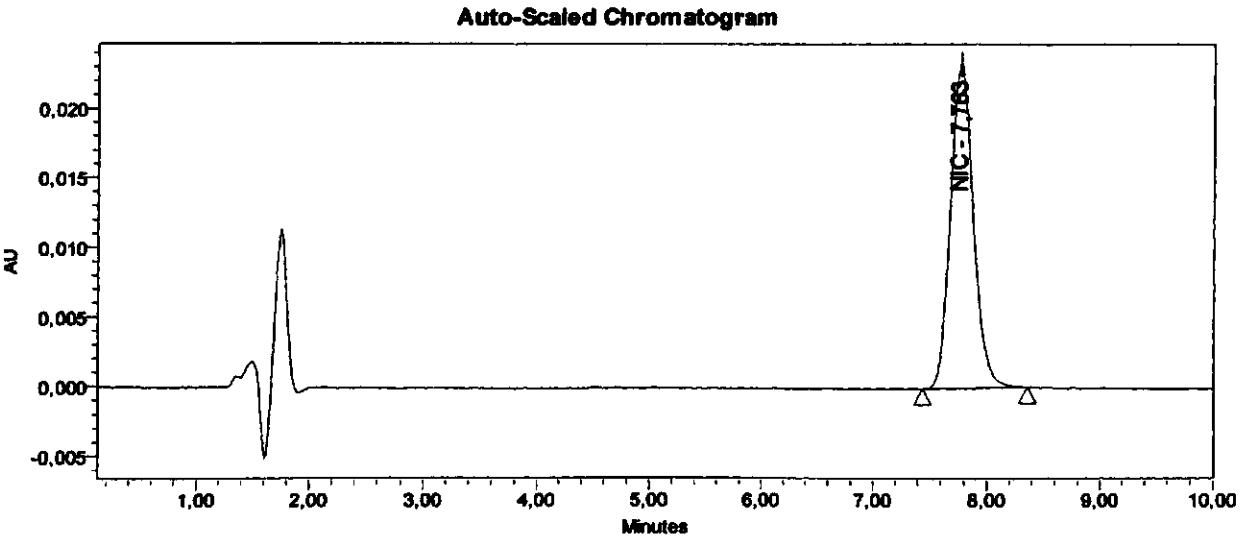
Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

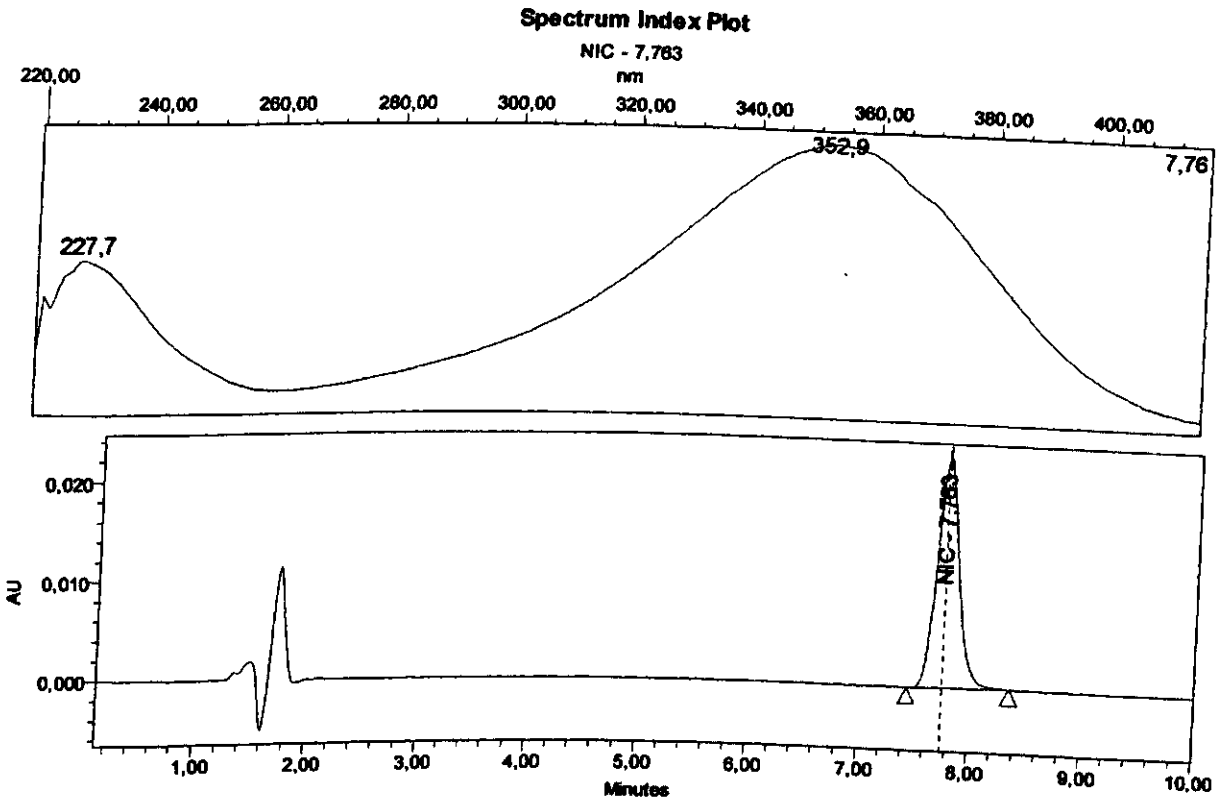
Recovery results:

- Percentage recovery: 99. %
- Single / duplicate determinations: ☒ single ☐ duplicate
- If duplicate, please give both percentages: ..... % and ..... %
- Spiking level: ..125. mg/kg



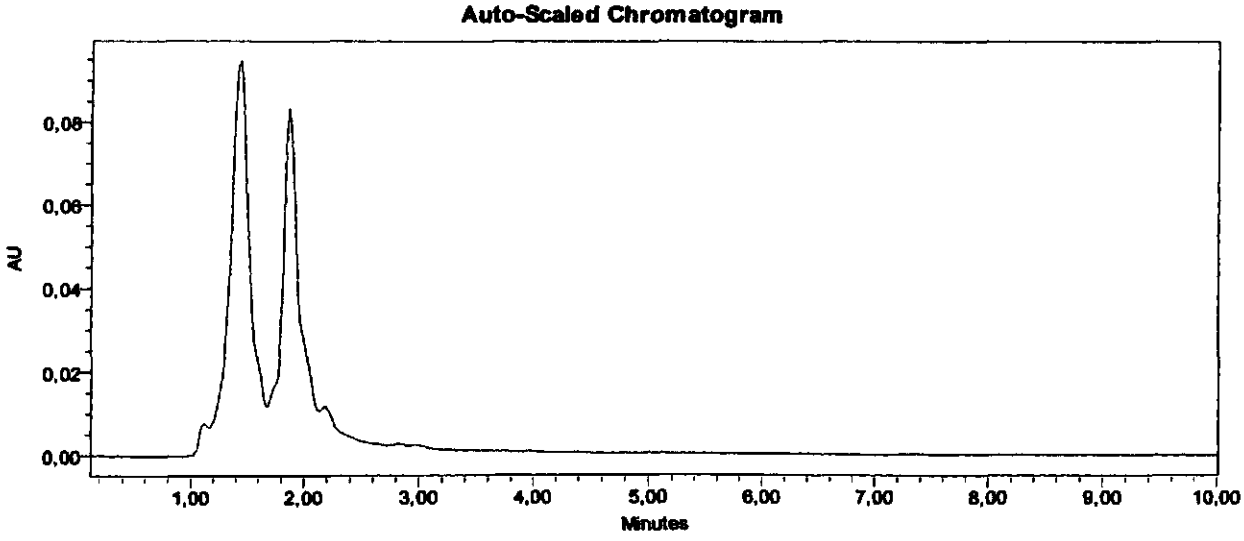
Peak Results

	SampleName	Name	RT	Area	Height	Amount	Units
1	7822/00	NIC	7,763	320406	23442	1,026	mg/kg



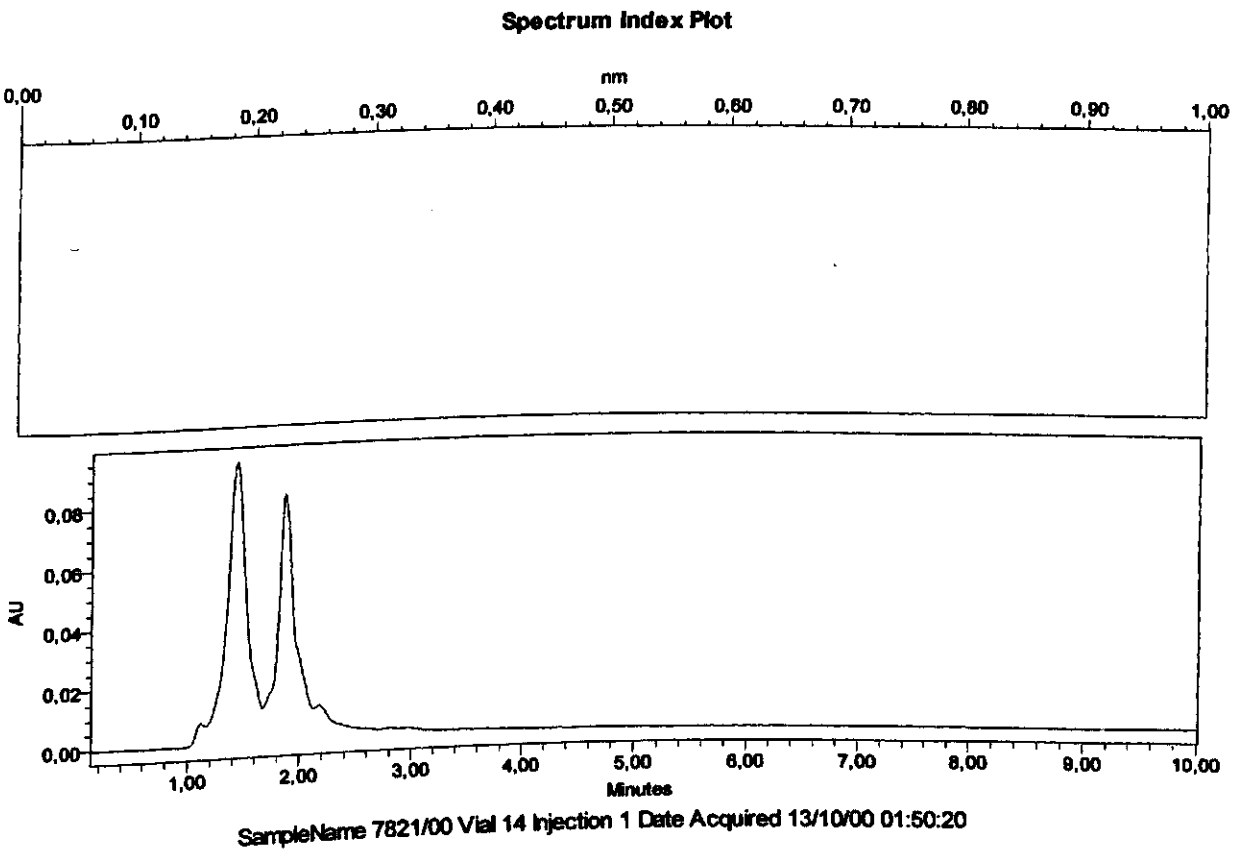
SampleName 7822/00 Vial 15 Injection 1 Date Acquired 13/10/00 02:01:20

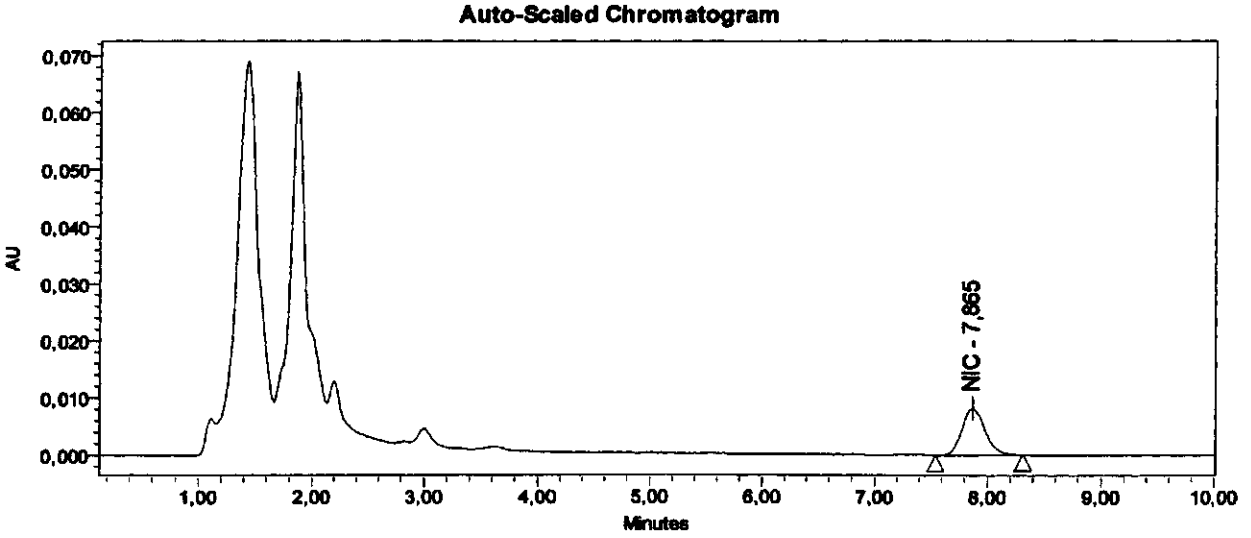
blank feed



Peak Results

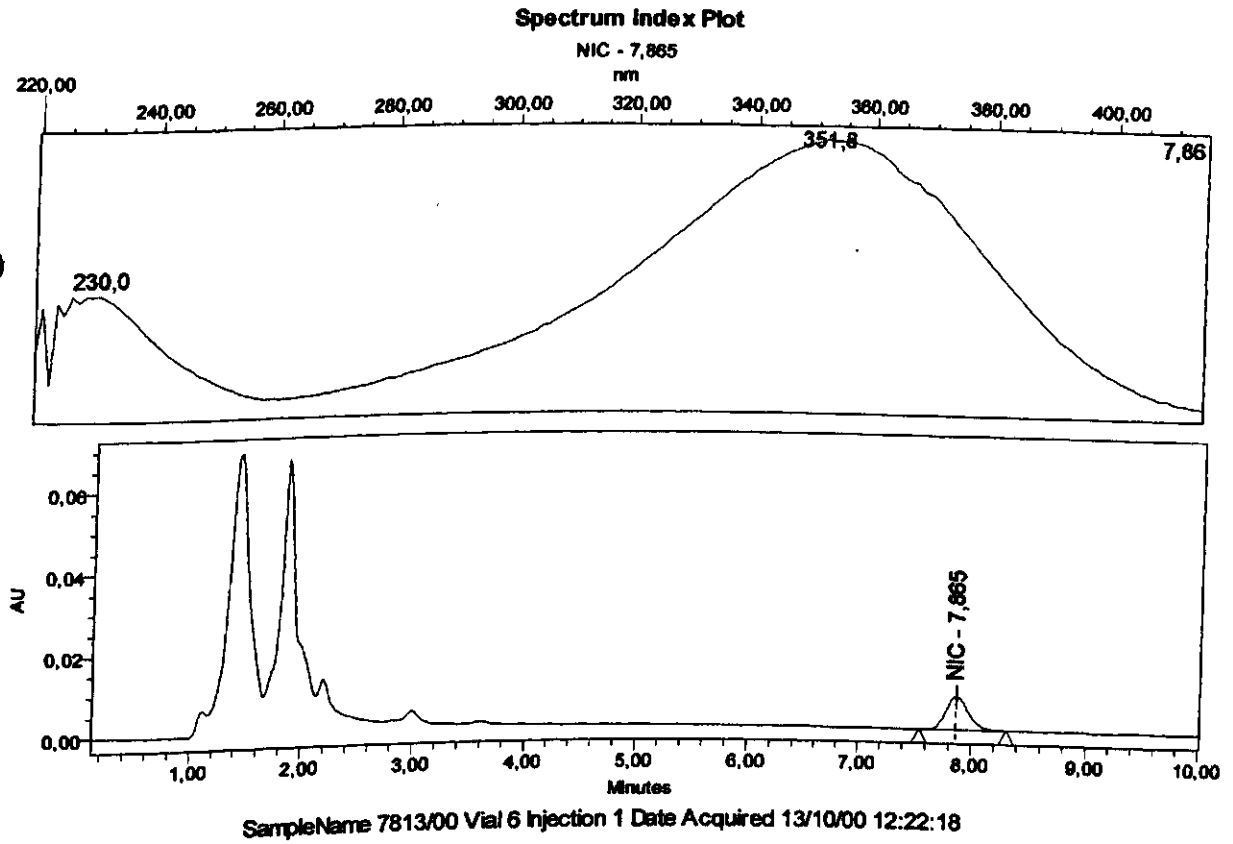
	SampleName	Name	RT	Area	Height	Amount	Units
1	7821/00	*NIC*	7,997				


Spectrum Index Plot
nm
0,00
0,10
0,20
0,30
0,40
0,50
0,60
0,70
0,80
0,90
1,00
AU
0,08
0,06
0,04
0,02
0,00
1,00
2,00
3,00
4,00
5,00
6,00
7,00
8,00
9,00
10,00
Minutes
SampleName 7821/00 Vial 14 Injection 1 Date Acquired 13/10/00 01:50:20



Peak Results

	SampleName	Name	RT	Area	Height	Amount	Units
1	7813/00	NIC	7,865	114038	8250	18,936	mg/kg



SampleName 7813/00 Vial 6 Injection 1 Date Acquired 13/10/00 12:22:18

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 35

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: Task 4 COLLABORATIVE STUDY
Lab-name:
Contact person:

e-mail:
fax:
telephone:

Date of analysis:

Analyte: NICARBAZIN

Sample code	Unit	Result (mg/kg)
354534		48
354546		24
354574		260
354581		41
354621		23
354651		2
354662		103
354678		123
354687		244
354690		1

Sample / Unit	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture	10930	11484

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Date(s) of analysis: 29-11-2000

Dilution factor of the samples:

- Feed samples (specify for which feed samples): 109→ 100, 3→ 10 (354574, 354687)
- Premixture: 0,5→ 100, 2,5→ 2 5

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ■ Other: Lichrospher 100-RP 18 5mm (125 x 4.0 mm)
- Mobile phase:
  - ☐ As described in the method
  - ■ Other: Methanol/water 700/300
- Flow-rate: 1,0 ml/min
- Injection volume: 20 µl
- Retention time of nicarbazin: 4,9 min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

Recovery results:

- Percentage recovery: 98,9 %
- Single / duplicate determinations: ☐ single ■ duplicate
- If duplicate, please give both percentages: 98,8% and 99,0%
- Spiking level: 100 mg/kg

Nicarbazin

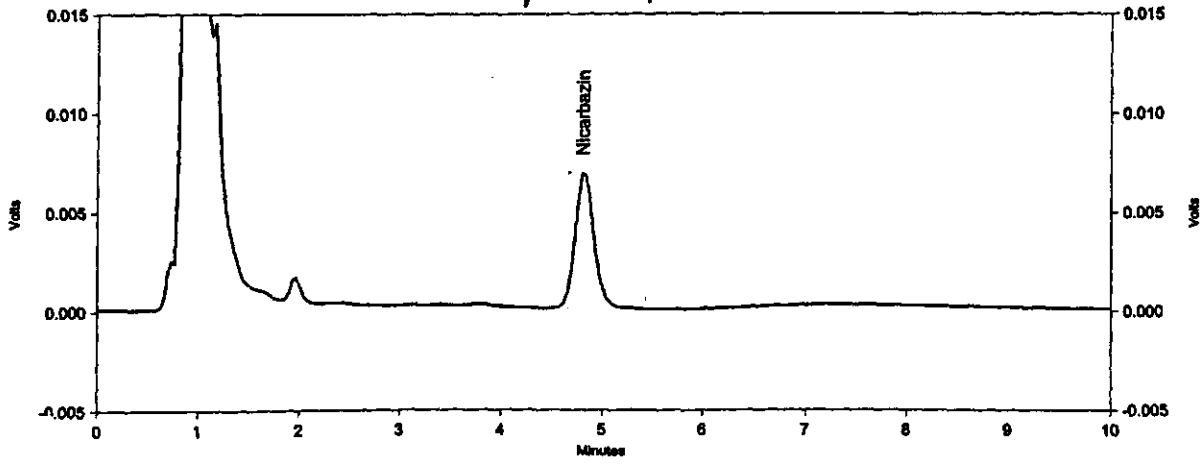
Monster: ? 3 450564

CANFAS code
354581

Instrument : UV_5
Methode : \\Fs_ \VOLI\DATA\Elite_Admin\Projects\Nicarbazin\Method\Nicarbazin.met
File : \\F\ \VOLI\DATA\Elite_Admin\Projects\Nicarbazin\Data\nicarbazin_291100_013
Sequence : \\Fs_ \VOLI\DATA\Elite_Admin\Projects\Nicarbazin\Sequence\nicarbazin_291100.seq
Gebruiker : asc
Runtijdstip : 11-29-2000 15:43:40

Inweeg : 2.5138
Verdunning : 100

Blind positive feed sample



UV-Detector Results

	Pk #	Retention Time	Area	Height	ESTD concentration	Units
Nicarbazin	1	4.80	87695	6828	40.64869	mg/kg

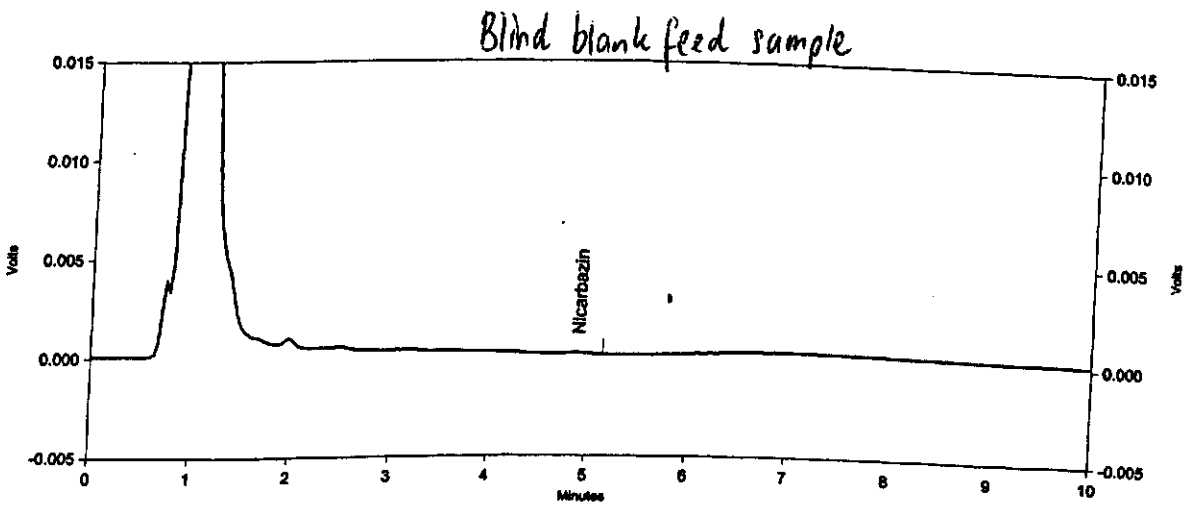
Nicarbazin

35

Monster: 1 450562

Instrument : 5
Methode : \VOLI\DATA\Elite_Admin\Projects\Nicarbazin\Method\Nicarbazin.met
File : \\Fs \VOLI\DATA\Elite_Admin\Projects\Nicarbazin\Data\nicarbazin_291100_011
Sequence : \\Fs_ \VOLI\DATA\Elite_Admin\Projects\Nicarbazin\Sequence\nicarbazin_291100.seq
Gebruiker : asc
Runtijdstip : 11-29-2000 15:22:42

Inweeg : 2.4974
Verdunning : 100



UV-Detector Results

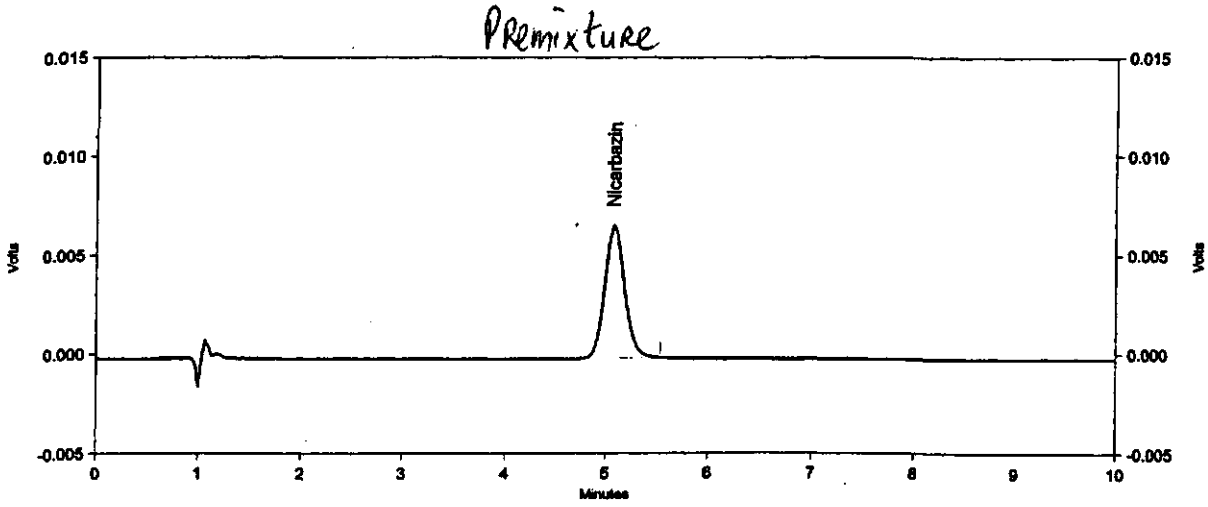
	Pk #	Retention Time	Area	Height	ESTD concentration	Units
Nicarbazin	1	4.90	740	60	0.34526	mg/kg

Nicarbazin

Monster: 13 450574 F 50

Instrument : UV_5
Methode : [illegible] .VOL1\DATA\Elite_Admin\Projects\Nicarbazin\Method\Nicarbazin.met
File : \Fs_ .VOL1\DATA\Elite_Admin\Projects\Nicarbazin\Data\nicarbazin_291100_045
Sequence : \Fs .VOL1\DATA\Elite_Admin\Projects\Nicarbazin\Sequence\nicarbazin_291100.seq
Gebruiker : asc
Runtijdstip : 11-29-2000 21:19:09

Inweeg : 0.4768
Verdunning : 5000



UV-Detector Results

	Pk #	Retention Time	Area	Height	ESTD concentration	Units
Nicarbazin	1	5.07	89494	6735	10891.40527	mg/kg

APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 38

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: Task 4 COLLABORATIVE STUDY
Lab-name:
Contact person:

e-mail:
fax:
telephone:

Date of analysis:

Analyte: NICARBAZIN

Sample code	Unit	Result (mg/kg)
384509		14,62
384512		93,03
384542		179,75
384560		0
384564		93,24
384579		181,6
384592		17,96
384633		0
384649		41,52
384674		35,57

Sample	Unit	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture		10242	10336

CANFAS COLLABORATIVE STUDIES - NICARBAZIN

Annex 4 – Questionnaire

Date(s) of analysis: **11/28/00**

Dilution factor of the samples:

- Feed samples (specify for which feed samples): **1,250 ± 0,001 g in 50 ml.**
- Premixture: **0,500 ± 0.001 g in 200 ml.**

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - X Other: **Hypersil ODS C-18, 250 x 4,6 mm, 5 µm**
- Mobile phase:
  - X As described in the method
  - ☐ Other:
- Flow-rate: **1** ml/min
- Injection volume: **20** µl
- Retention time of nicarbazin: **5** min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank samples
- Premixture

Please indicate the nicarbacin peak with an arrow

Recovery results:

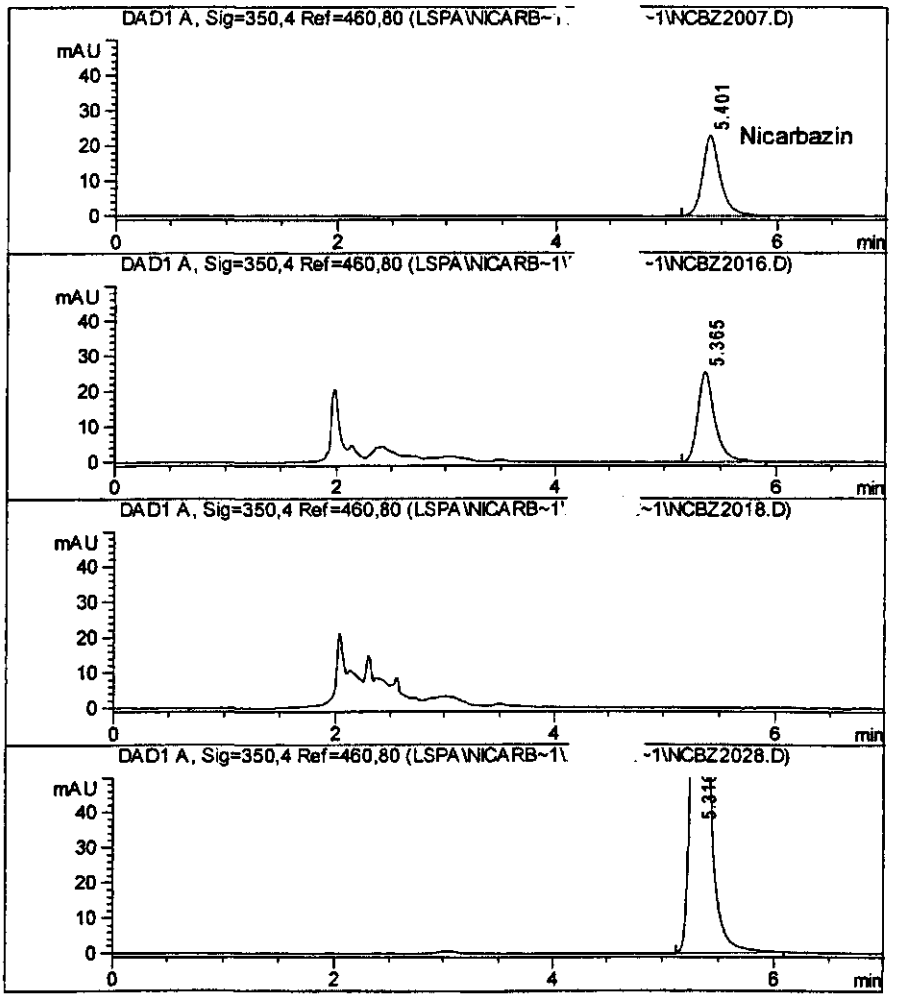
- Percentage recovery: **83 %**
- Single / duplicate determinations: **X** single ☐ duplicate
- If duplicate, please give both percentages: ... % and ... %
- Speaking level: **125** mg/kg

CANFAS COLLABORATIVE STUDIES - NICARBAZIN

Remarks / Comments (if necessary, continue on another page):

Two calibration ranges has been used for quantification of samples. First (0,25 – 5 ppm; n = 8) for feed samples and second (10 – 50 ppm; n= 5) for premixture.

Chromatograms for standard (4 ppm), sample (384542), blank and premixture



APPENDIX 6

Table with results, questionnaire (page 1) and chromatograms

of partner 39

CANFAS

Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: Task 4 COLLABORATIVE STUDY
Lab-name:
Contact person:

e-mail:
fax:
telephone:

Date of analysis:

Analyte: NICARBAZIN

Sample code	Unit	Result (mg/kg)
394588		20,7
394617		114,3
394620		259,2
394637		264,2
394643		117,2
394654		< 2
394665		17,2
394669		44,1
394672		< 2
394681		42,5

Sample / Unit	Result 1 (mg/kg)	Result 2 (mg/kg)
Premixture	10194	10453

CANFAS COLLABORATIVE STUDIES OCTOBER 2000 - NICARBAZIN

Annex 4 - Questionnaire

Date(s) of analysis: 23-11-2000

Dilution factor of the samples:

- Feed samples (specify for which feed samples): all feed sample : 100 x (volumetric flask of 100 ml)
- Premixture: 2500 x (volumetric flask of 100 ml + 25 x)

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ■ Other: 125 x 3 mm, Lichrospher RP 18, 5 µm, Merck 51232.
- Mobile phase:
  - ■ As described in the method
  - ☐ Other: .....
- Flow-rate: 0.5 ml/min
- Injection volume: 25 µl
- Retention time of nicarbazin: 3.5 min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample
- Premixture

Please indicate the nicarbazin peak with an arrow

Recovery results:

- Percentage recovery: 102 %
- Single / duplicate determinations: ■ single ■ duplicate
- If duplicate, please give both percentages: 102 % and 102 %
- Spiking level: 12.5 mg/kg

positive FEED SAMPLE

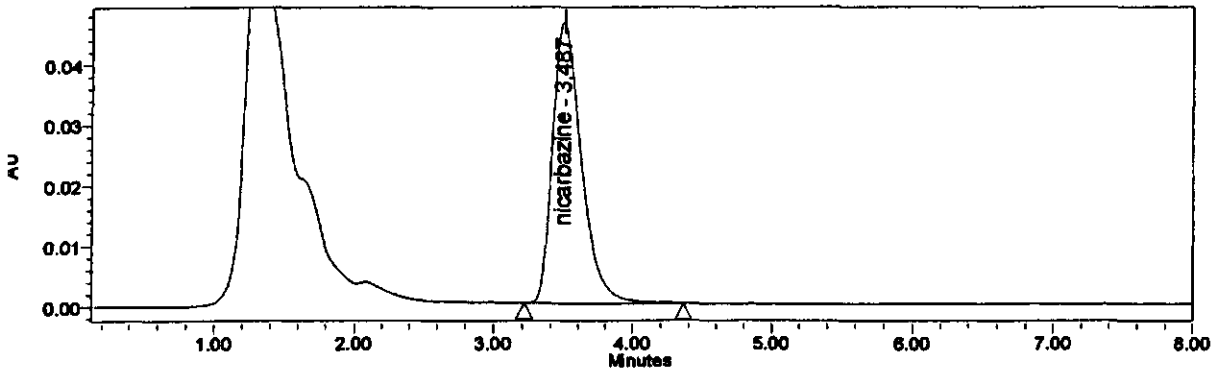
M. 39617

39

Sample Information

SampleName	20001006.015 5	Sample Type	Unknown
Vial	7	Date Acquired	11/23/2000 2:34:25 PM
Injection	1	Acq Method Set	Meth_nic_voer_hoog
Injection Volume	25.00 ul	Processing Method	Proc_meth_nic_voer_hoo
Channel	996	Date Processed	11/24/2000 8:16:11 AM
Run Time	8.0 Minutes		

Auto-Scaled Chromatogram



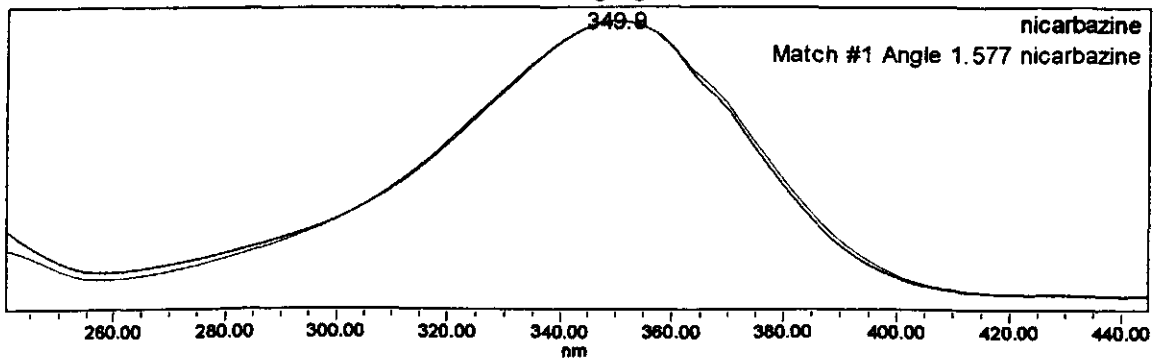
Nicarbazine piek resultaten

	Name	RT	Area	Height	Amount	Units
1	nicarbazine	3.487	674639	48534	114.306	mg/kg

Nicarbazine PDA resultaten

	Name	RT	Purity1 Angle	Purity1 Threshold	Match1 Angle	Match1 Threshold	Match1 Spect. Name	Match1 Lib. Name
1	nicarbazine	3.487	0.324	1.787	1.577	1.165	nicarbazine	nic voer

Bevestiging



Vial 7 SampleName 20001006.015 5 Date Acquired 11/23/2000 2:34:25 PM

BLANK FEED SAMPLE

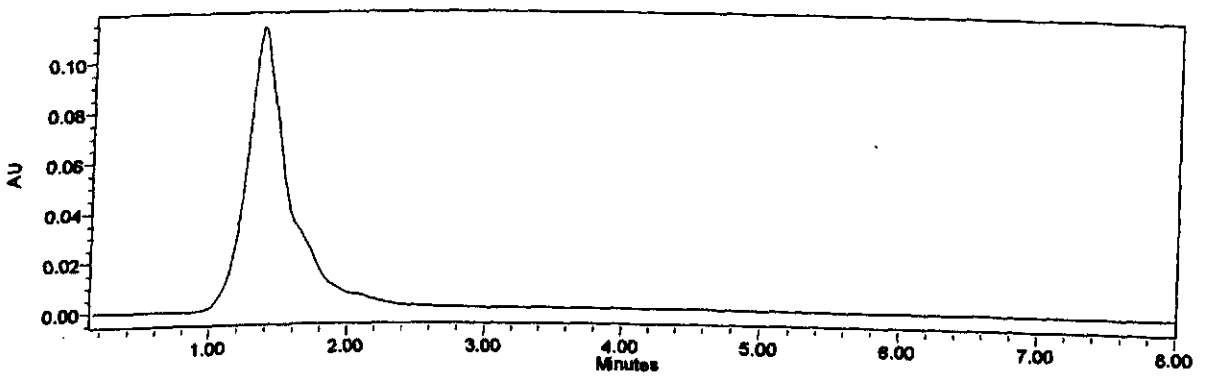
Current Date 11/24/2000

39

Sample Information

SampleName	20001006.019 13	Sample Type	Unknown
Vial	13	Date Acquired	11/23/2000 3:28:13 PM
Injection	1	Acq Method Set	Meth_nic_voer_hoog
Injection Volume	25.00 ul	Processing Method	Proc_meth_nic_voer_hoo
Channel	996	Date Processed	11/24/2000 8:16:55 AM
Run Time	8.0 Minutes		

Auto-Scaled Chromatogram



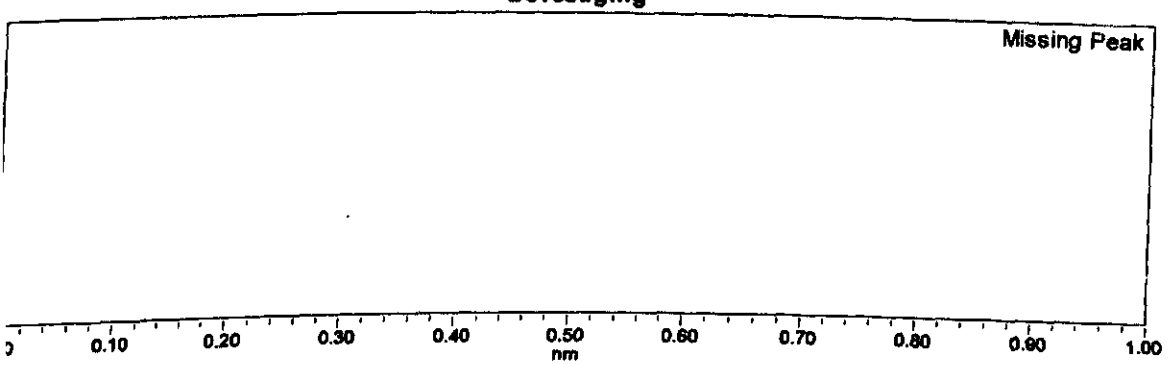
Nicarbazine piek resultaten

	Name	RT	Area	Height	Amount	Units
1	nicarbazine	3.500				

Nicarbazine PDA resultaten

	Name	RT	Purity1 Angle	Purity1 Threshold	Match1 Angle	Match1 Threshold	Match1 Spect. Name	Match1 Lib. Name
1	nicarbazine	3.500						

Bevestiging



Vial 13 SampleName 20001006.019 13 Date Acquired 11/23/2000 3:28:13 PM

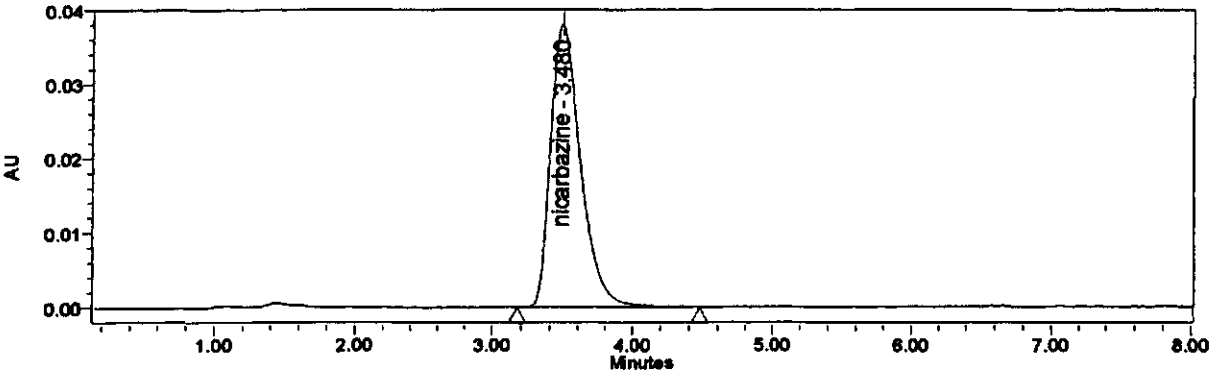
PREMIXTURE

39

Sample Information

SampleName	20001006.024 27	Sample Type	Unknown
Vial	24	Date Acquired	11/23/2000 5:06:57 PM
Injection	1	Acq Method Set	Meth_nic_voer_hoog
Injection Volume	25.00 ul	Processing Method	Proc_meth_nic_voer_hoo
Channel	996	Date Processed	11/24/2000 8:18:30 AM
Run Time	8.0 Minutes		

Auto-Scaled Chromatogram



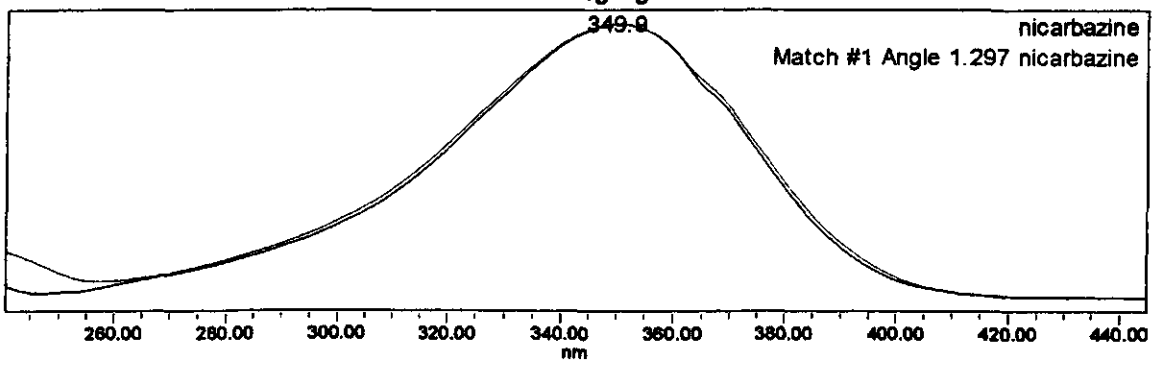
Nicarbazine piek resultaten

	Name	RT	Area	Height	Amount	Units
1	nicarbazine	3.480	566212	38008	10194.055	mg/kg

Nicarbazine PDA resultaten

	Name	RT	Purity1 Angle	Purity1 Threshold	Match1 Angle	Match1 Threshold	Match1 Spect. Name	Match1 Lib. Name
1	nicarbazine	3.480	0.300	1.493	1.297	1.101	nicarbazine	nic voer

Bevestiging



Vial 24 SampleName 20001006.024 27 Date Acquired 11/23/2000 5:06:57 PM