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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)

Co-ordinator: Dr. J. de Jong

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CANFAS - 2nd Collaborative study for the determination of olaquindox in feedingstuffs by HPLC

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SUMMARY

This report describes the results of the 2nd collaborative study of an HPLC method for the growth promoter olaquindox in two piglet feeds. 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).

In the first collaborative study many laboratories reported difficulties with the practicability of the method due to the low ratio between the volume of extraction solvent (50 ml) and the weight of feed (25 g). For this reason the CANFAS-method was modified in such a way that the ratio between the extraction volume and the sample weight was increased to 5. A second round of collaborative studies for final validation of the method was organised.

The principle of the method is as follows: The sample is extracted by a mixture of water - methanol. The content of olaquindox is determined by reversed-phase high-performance liquid chromatography (HPLC) with UV-detection at 380 nm.

The samples that were tested in the collaborative study were 2 piglet feeds with declared olaquindox contents of 2,5 mg/kg and 10 mg/kg respectively. The feed samples were sent to the participants as blind duplicates. The participants were asked to do duplicate determinations per sample.

Results were reported by 22 laboratories. Statistical evaluation was performed according to ISO 5725. The results show that with the modified method acceptable results are obtained for repeatability (rd, < 10 %) and reproducibility (Horrat ratios < 2).

During the first collaborative study blank samples were analysed: no interfering substances were detected, so the results obtained for the blank feed were acceptable.

Acceptable results were obtained for recovery, reported values ranged between 52 and 107%.

The final method can be recommended for adoption as an official method and together with the results of the collaborative study it will be sent to the European Commission (CEMA), CEN and ISO.
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), the official EC-method for olaquindox (Directive 98/64/EC) has been validated for low contents in feeds. Olaquindox is a growth promoter that was registered for use in feeds for piglets with contents ranging from 15 - 50 mg/kg (50 - 100 mg/kg for milk replacers). Since September 1999, the use of olaquindox as a feed additive is banned in the EU. In order to allow adequate control of possible illegal use, the objective was to validate the official EC-method (an HPLC method with UV-detection) for contents 5 - 10 times lower than the lowest content formerly permitted, viz. down to 1,5 mg/kg.

The method was validated by LUFA - Augustenberg, Karlsruhe, Germany. Compared with the original method, the ratio between the extraction volume and the sample weight was modified: in the original method this ratio was 10; in order to increase the limit of detection, in the modified method this ratio was decreased to 2 (see report K. Michels, Final report on evaluation of method validation for olaquindox and carbadox in feeds at low contents, 01-11-1999).

Subsequently, the method was subjected to between-lab validation by the State Laboratory, Dublin, Ireland (see report P. Shearan, January 2000) and Istituto Superiore di Sanita (I.S.S.), Roma, Italy (see report G. Brambilla, January 2000). In general, the criteria as described in the amended Project Plan are fulfilled. The recoveries are often lower than 80 % (down to 60 %) but, while the use of olaquindox has been forbidden, this is not regarded as a major shortcoming (see Second Annual Report CANFAS, J. de Jong, 12-08-2000).

Prior to the first 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 olaquindox. Also prior to the production of the materials for the collaborative study, separate batches of the materials had been produced for homogeneity and stability testing. The between- and within-sample homogeneity was satisfactory and the results showed that olaquindox is stable in the feeds at room temperature during a period of 4 months (see Second Annual Report CANFAS, J. de Jong, 12-08-2000).

In the first collaborative study many laboratories reported difficulties with the practicability of the method due to the low ratio between the volume of extraction solvent (50 ml) and the weight of feed (25 g).

During the evaluation meeting organised after the first collaborative study, it was decided to modify the CANFAS method in such a way that the ratio between the extraction volume and the sample weight was increased to 5. A second round of collaborative studies for final validation of the method was organised.

The samples that were prepared for the collaborative study were two piglet feeds with declared olaquindox contents of 2,5 and 10 mg/kg respectively. The feed samples were sent to the participants as blind duplicates. Before these samples were shipped, the between sample homogeneity of the feed samples containing olaquindox 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 2nd collaborative study.
2 PARTICIPANTS

The following laboratories/persons participated in the collaborative study.

- Administration des Services Technique de l’Agriculture Division des Laboratoires, Ettelbruck, Luxemburg; C. Strottner
- Bundesamt und Forschungszentrum für Landwirtschaft (BFL), Wien, Austria; B. Stoisser, M. Wieshaider
- INETI/DTIA, Lisbon, Portugal; I. Felgueiras, C. Saldanha
- 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
- Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy; G. Biancotto, B. Allegretta
- Istituto Zooprofilattico Sperimentale delle regioni Lazio e Toscana, Roma, Italy; A. Ubaldi, A. di Lullo.
- Laboratoire Inter Régional DGCCRF, Rennes, France; C. Genouel, M.C. Rues, M. Joubert.
- Laboratorio Arbitral Agroalimentario, Madrid, Spain; D.A. Pons, J. Muñoz
- 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; J. Cowles
- LUFA – Augustenburg, Karslruhe, Germany; K. Michels, S. Witzemann.
- LUFA-ITL Kiel, Kiel, Germany; H. Wehage, H. Graepel
- Masterlab, Putten, The Netherlands; K. van Schalm, A. Schaaf.
- National Veterinary Institute, Uppsala, Sweden; E. Nordkvist, A. Stepinska
- Plant Production Inspection Centre Agricultural Chemistry Department, Vantaa, Finland; R. Muhonen, Y. Hyvönen
- Rijksontledingslaboratorium, Tervuren, Belgium; K. Haustraete, A. Fontaine, M. Lekens, R. van Sandt
- RIKILT, Wageningen, The Netherlands; H. Kleijn, H. van der Kamp
- State Laboratory Dublin, Ireland, P. Shearan
- Universität Hohenheim, Landesanstalt für Landwirtschaftliche Chemie, Stuttgart, Germany; K. Schwadorf, A. Eschle
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

<table>
<thead>
<tr>
<th>Type of feed</th>
<th>Declared content</th>
<th>Units</th>
<th>Subcontractor</th>
<th>Date of production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piglet feed</td>
<td>2.5</td>
<td>mg/kg</td>
<td>IPC - Dier, Barneveld (NL)</td>
<td>25-09-2001</td>
</tr>
<tr>
<td>Piglet feed</td>
<td>10</td>
<td>mg/kg</td>
<td>IPC - Dier, Barneveld (NL)</td>
<td>25-09-2001</td>
</tr>
</tbody>
</table>

The complete composition of the feeds is given in Appendix 2 (in Dutch). The main composition of the two feeds is given in Table 2.

Table 2: Main composition of the two feeds

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>18.1</td>
</tr>
<tr>
<td>Crude fat</td>
<td>4.3</td>
</tr>
<tr>
<td>Starch</td>
<td>39.3</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>4.4</td>
</tr>
<tr>
<td>Crude ash</td>
<td>5.9</td>
</tr>
<tr>
<td>Moisture</td>
<td>12.3</td>
</tr>
</tbody>
</table>

The composition of the feed, with regards to the ingredients, was the same as of the feeds that were produced by IPC-Dier in September 1999 for stability testing (see Report on homogeneity and stability studies of samples for the collaborative studies for olaquindox, K. Michels, LUFA Augustenberg, Germany, 05/05/2000) and in September 2001 for the first collaborative study (see report of first collaborative study see RIKILT report 2002.014). The composition of the feeds, in terms of crude protein, fat, etc, was nearly the same. In the produced feeds for the second round of collaborative study the crude ash content is somewhat higher (5.9% - 4.7%). The feed products have been prepared in a quantity of 500 kg each. To achieve a maximum degree of homogeneity halfway through the production 54 kg of feed are withdrawn from the stream for subsampling activities and put into three 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. All subsamples have been stored at room temperature (ca. 20 °C).

3.1.2 Sample homogeneity
The homogeneity of the samples was studied by LUFA Augustenberg 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 are attached in Appendix 3. Table 3 gives a summary of these results.

Table 3: Results of homogeneity tests for olaquindox in piglet feeds

<table>
<thead>
<tr>
<th>Results</th>
<th>Declared content (mg/kg)</th>
<th>Measured content (mg/kg)</th>
<th>Homogeneity results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piglet feed</td>
<td>2,5</td>
<td>2,90</td>
<td>Between sample CV (%)</td>
</tr>
<tr>
<td>Piglet feed</td>
<td>10</td>
<td>9,90</td>
<td>Not determined</td>
</tr>
</tbody>
</table>

According to the Project Plan the CV’s for homogeneity should not exceed 2 times the CV’s for repeatability (CV_{hom} < 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 16 %. Both between sample CV’s fulfil these requirements. Thus, it is concluded that the samples are sufficiently homogeneous.

3.1.3 Sample logistics
The samples were sent as blind duplicates. The codes are given in Appendix 4. The samples were sent to the participants by courier service on 2 November 2001 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 Dr. A. Plöger, Danish Plant Directorate, Lyngby (DK). According to the specifications (see Report 2002.014), the purity of the reference standard (Lot Nr. 890416) is 99.46 %.

The expiration date of the reference standard was April 2001. The identity and content was checked by RIKILT. The identity could be confirmed by UV, ^1H-NMR as well as mass spectrometry. The purity was determined by ^1H-NMR and UV spectroscopy and was shown to be approx. 100 % (see Report 2002.014).

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 following columns were recommended in the method:
- Hypersil ODS 5 μm, 200 x 4,6 mm;
- Spherisorb ODS-2 5 μm, 250x4,6 mm;
- LUNA C18(2) 250 x 4,6 mm.

The mobile phase described in the method is a mixture of water and methanol 900:100 (v/v). 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 scrutiny of results for consistency and outliers was checked by:
- Graphical consistency techniques: Mandel's h plot for between-laboratory variability, Mandel's k plot for within-laboratory variability
- 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

<table>
<thead>
<tr>
<th>Partner</th>
<th>Column</th>
<th>Mobile phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Tracer extrasil ODS 5x25x0,46</td>
<td>As described in the method</td>
</tr>
<tr>
<td>15</td>
<td>Inertsil ODS-2; 5 μm; 250 x 2,6 mm</td>
<td>As described in the method</td>
</tr>
<tr>
<td>16</td>
<td>Phenomenex LUNA C18 (2); 5 μm; 150 x 4,6 mm</td>
<td>As described in the method</td>
</tr>
<tr>
<td>17</td>
<td>Sperisorb S10 ODS-1; 10 μm</td>
<td>As described in the method</td>
</tr>
<tr>
<td>18</td>
<td>Sperisorb ODS-2; 5 μm; 150 x 4,6 mm</td>
<td>As described in the method</td>
</tr>
<tr>
<td>20</td>
<td>ODS Hypersil C18; 5 μm; 200 x 4,6 mm</td>
<td>As described in the method</td>
</tr>
<tr>
<td>21</td>
<td>Supelcosil LC18; 25 cm x 4,6 mm + supelguard LC18; 2 cm x 4,6 mm</td>
<td>Acetonitril: acetate buffer (0,01 M; pH 4,6) Gradient elution</td>
</tr>
<tr>
<td>22</td>
<td>Hypersil C18 ODS BDS; 5 μm; 250 x 4,6 mm</td>
<td>As described in the method</td>
</tr>
<tr>
<td>23</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>24</td>
<td>Waters C18; 5 μm; 250 x 4,6 mm</td>
<td>As described in the method</td>
</tr>
<tr>
<td>25</td>
<td>RP C18 Lichrocart; 5 μm; 250 mm x 4 mm (Merck)</td>
<td>Phosphate buffer(0,0 M; pH 2,8): Acetonitrile Gradient elution</td>
</tr>
<tr>
<td>26</td>
<td>Spherisorb ODS 2; 5 μm; 250 mm x 4,6 mm</td>
<td>As described in the method</td>
</tr>
<tr>
<td>29</td>
<td>Nova-Pak C18; 4 μm; 4,6 x 250 mm</td>
<td>As described in the method</td>
</tr>
<tr>
<td>31</td>
<td>As described in the method</td>
<td>As described in the method</td>
</tr>
<tr>
<td>32</td>
<td>Lichrospher RP-Select B; 5 μm; 250x4 mm</td>
<td>As described in the method</td>
</tr>
<tr>
<td>33</td>
<td>As described in the method</td>
<td>As described in the method</td>
</tr>
<tr>
<td>34</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>35</td>
<td>As described in the method</td>
<td>As described in the method</td>
</tr>
<tr>
<td>37</td>
<td>Lichrosper RP18-5 endcapped ; 25 x 4 mm</td>
<td>As described in the method</td>
</tr>
<tr>
<td>38</td>
<td>Symetry C-18; 3,5 μm; 150 x 2,1 mm</td>
<td>Isocratic methanol/water 5:95</td>
</tr>
<tr>
<td>40</td>
<td>C18 sperical; 5 μm; 3,9 x 150 mm</td>
<td>As described in the method</td>
</tr>
<tr>
<td>41</td>
<td>As described in the method</td>
<td>As described in the method</td>
</tr>
</tbody>
</table>
5 RESULTS

For each participant the reported results for the samples, the completed questionnaire and representative chromatograms are annexed in Appendix 5.

5.1 Statistical evaluation

Originally laboratory 12 reported results that were not in agreement with the results of the other participants and that deviated much from the theoretical olaquindox concentrations. The reported results from lab 12 were 0.50, 0.53, 0.53, 0.53 mg of olaquindox/kg for the sample with a declared content of 2.5 mg/kg and 1.99, 2.04, 1.99, 2.03 mg of olaquindox/kg for the sample with a declared content of 10 mg/kg. Due to the magnitude of the deviations it was most likely that the results would cause outliers on both levels. Lab 12 was contacted to try to ascertain the cause of the discrepant behaviour. According to the explanation this lab had met problems with the solubility of the reference standard, because they had prepared a stock standard solution more concentrated than the one indicated in the method. After repetition of the analysis by following exactly the procedure as described lab 12 reported new values. Based on the findings mentioned above it was decided to accept the new results.

The results reported by the participants are given in Table 6.

Statistical analysis shows that the results of the laboratories do not contain Cochran or Grubbs' outliers or stragglers. The values for the statistical parameters (mean, relative standard deviations for repeatability and reproducibility) are given in Table 6. According to the Project Plan, the rsd\(_R\)-values should be \(\leq 10\%\). For both samples this criterion is met and consequently it can be concluded that the repeatability is satisfactory.

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 both samples this criterion is met and established rsd\(_{R}\)-values are in line with values predicted by the Horwitz equation. Consequently it can be concluded that the reproducibility of the changed method is satisfactory.

Table 5: Horratt ratios of the olaquindox collaborative study

<table>
<thead>
<tr>
<th>Mean</th>
<th>Predicted rsd(_R)</th>
<th>Established rsd(_R)</th>
<th>Horratt(^1)</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.47</td>
<td>14,0</td>
<td>18,5</td>
<td>1.33</td>
<td>Reproducibility OK</td>
</tr>
<tr>
<td>8.79</td>
<td>11,5</td>
<td>13,1</td>
<td>1.13</td>
<td>Reproducibility OK</td>
</tr>
</tbody>
</table>

\(^{1}\) = Horratt is the ratio between the established rsd\(_{R}\) and the predicted rsd\(_{R}\)

The Mandel h and k plots are shown in Figure 1.
Table 6: Results reported by the participants.

<table>
<thead>
<tr>
<th>Lab</th>
<th>Sample</th>
<th>OLA2 2.5 ppm</th>
<th>OLA2 2.5 ppm</th>
<th>OLA2 2.5 ppm</th>
<th>OLA2 10 ppm</th>
<th>OLA2 10 ppm</th>
<th>OLA2 10 ppm</th>
<th>OLA2 10 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>2.5 ppm</td>
<td>1.90</td>
<td>2.29</td>
<td>1.71</td>
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<td>15</td>
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<td>1.88</td>
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<td>7.54</td>
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<td>7.82</td>
<td>7.82</td>
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<td>2.48</td>
<td>9.85</td>
<td>9.85</td>
<td>9.85</td>
<td>9.85</td>
</tr>
</tbody>
</table>

| number of labs | 22 |
| m (mg/kg)      | 2.47 |
| rsd (\%)       | 6.56 |
| rsdR (\%)      | 18.5 |
Figure 1: Mandel h and k plots of results reported by the participants.
5.2 Recoveries

Table 7: Recoveries

<table>
<thead>
<tr>
<th>Partner</th>
<th>Spiking level (mg/kg)</th>
<th>Recovery 1 in %</th>
<th>Recovery 2 in %</th>
<th>Average recovery in (%)</th>
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<tbody>
<tr>
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<td>25</td>
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</tr>
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<td>22</td>
<td>3.2/6.3</td>
<td>105</td>
<td>97</td>
<td>101</td>
</tr>
<tr>
<td>23</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
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<td>88</td>
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<td>90</td>
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<td>41</td>
<td>2.5</td>
<td>101</td>
<td>99</td>
<td>100</td>
</tr>
</tbody>
</table>

Recoveries range from 52 - 107 %. This range is broader than the range (60 - 90 %) that was measured in the between-lab validation of the method (see Second Annual Report CANFAS, J. de Jong, 12-08-2000).

Although the mean recovery value reported by lab 38 (53 %) is low, it is not a Grubbs outlier or a straggler.
5.4 Remarks

Table 8: Remarks made by the partners

<table>
<thead>
<tr>
<th>Partner</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>No remarks</td>
</tr>
<tr>
<td>15</td>
<td>No remarks</td>
</tr>
<tr>
<td>16</td>
<td>ad 3.5.1.: Concentration of Olaquindox stock standard solution was 36.5 µg/ml; ad 3.5.2.: Concentration of the corresponding calibration solutions: 0.365; 0.730; 1.825; 3.650 and 7.300 µg/ml ad 5.3.2.: Linear regression calculated: 0.99999 ad 5.2: Extraction: the following parameters were used: - Centrifugation 10 minutes with 7000 rpm (instead of filtration step) - The supernatant liquids were additionally filtered by using membrane filters (Machery&amp;Nagel, Chromafil Type A-45/25, 0.45 µm)</td>
</tr>
<tr>
<td>17</td>
<td>No remarks</td>
</tr>
<tr>
<td>18</td>
<td>HPLC equipment: pump, autosampler, column oven = HP1050; DAD = HP1100 Slight modifications: - ad 3.5.1.: Stock standard solution = 50 mg, weigh to the nearest 1 mg, in 2000 ml water. - ad 3.5.2.: 1.25 µg/ml standard solution = 5 ml diluted to 100 ml (instead 2.5 ml to 50 ml) - Samples stored at refrigerated temperature until analysis (&lt;8 °C). - Filtration and centrifugation of the extracts.</td>
</tr>
<tr>
<td>20</td>
<td>No remarks</td>
</tr>
<tr>
<td>21</td>
<td>We centrifuged 10 ml of the final extract instead of filtering the whole extract on a paper filter. Then we filtered 2 ml of the centrifuged extract on an Acrodisc filter (0.45 µm) before HPLC analysis.</td>
</tr>
<tr>
<td>22</td>
<td>The Olaquindox content was calculated from the peak area by reference to the calibrations graph.</td>
</tr>
<tr>
<td>23</td>
<td>Not reported</td>
</tr>
<tr>
<td>24</td>
<td>The extraction step is improved if compared to the first edition of this method. Still, centrifugation is necessary as well as filtration through 0.45 µm just before HPLC injection.</td>
</tr>
<tr>
<td>25</td>
<td>Column overpressure recorded after repeated injections with RT not constant. It is suggested to reduce flow rate to 1 ml/min with a slight increase of the organic phase. As alternative we suggest a gradient elution able to clean the column.</td>
</tr>
<tr>
<td>Partner</td>
<td>Remarks</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>26</td>
<td>The procedure was well documented and straightforward to follow. We have had one major problem with retention time stability of olaquindox. Initial injections of all standards and pre-injections (to verify system stability) all gave excellent response, Rt was 9.9 min. The blank sample was then injected and gave a zero response at the Rt of olaquindox. However, after this time the Rt of olaquindox reduced to between 9.0 and 9.2 minutes, but the signal response did not change. Initially we thought that this may have been a temperature effect as we run the samples overnight and we know that the laboratory temperature rises when the air conditioning is switched off. We therefore re-extracted the samples and put the LC-column in an oven at 35 °C and reduced the flow rate to minimise these effects. However, it made no difference.</td>
</tr>
<tr>
<td>29</td>
<td>No remarks</td>
</tr>
<tr>
<td>31</td>
<td>No remarks</td>
</tr>
<tr>
<td>32</td>
<td>No remarks</td>
</tr>
<tr>
<td>33</td>
<td>No remarks</td>
</tr>
<tr>
<td>34</td>
<td>No remarks</td>
</tr>
<tr>
<td>35</td>
<td>No remarks</td>
</tr>
<tr>
<td>37</td>
<td>The method is now easier to manipulate using the modifications in Annex I. We carried out the entire method in glass centrifuge tubes. i) flat bed shaker used: these tubes were put horizontal on bed - effective shaking/mixing noted. ii) after shaking the tubes were placed in centrifuge for 5 min. Therefore no need to use GFA filters. Extracts were filtered prior to LC. LC-conditions: working at high psi: 1 ml/min ~ 2800 psi</td>
</tr>
<tr>
<td>38</td>
<td>We have used two different feed samples from our collection as blank feed for recovery purposes. They do not belong to the other CANFAS Collaborative feed samples because we spent all of them. So, one sample is lamb feed and the other one is a piglet feed. Both of them had got a similar aspect to the CANFAS Collaborative II feed samples. We have observed that recovery and blank samples make spherical clusters (lump) after addition of olaquindox standard solution in water. These lumps were not broken after addition of methanol.</td>
</tr>
<tr>
<td>40</td>
<td>No remarks</td>
</tr>
<tr>
<td>41</td>
<td>No remarks</td>
</tr>
</tbody>
</table>
From the results it can be concluded that with the modified method acceptable results are obtained for repeatability (rsd, < 10 \%) and reproducibility (Horrat ratios < 2).

During the first collaborative study blank samples were analysed: no interfering substances were detected, so the results obtained for the blank feed were acceptable.

Reported values for recovery ranged between 52 and 107\%. The recoveries are sometimes lower than 80 \% (down to 52 \%) but, while the use of olaquindox has been forbidden, this is not regarded as a major shortcoming (see Second Annual Report CANFAS, J. de Jong, 12-08-2000).

The remarks made by the participants indicate that no difficulties were encountered. Some laboratories applied centrifugation of the samples instead of filtration. According to the method description this alternative may be applied.

The final method can be recommended for adoption as an official method and together with the results of the collaborative study it will be sent to the European Commission (CEMA), CEN and ISO.
ACKNOWLEDGEMENTS

Financial support from the European Commission, DG Research, Standards, Measurements and Testing Programme (SMT) is gratefully acknowledged.
Dr. A. Ploeger, Danish Plant Directorate is thanked for supplying the olaquindox 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)
Participants CANFAS collaborative study Olaquindox

Dear colleague,

As agreed at the CANFAS evaluation meeting June 19th, 2001 at Tervuren a second round of collaborative study for olaquindox has to be organised. We appreciate your willingness to participate very much. Together with this letter you will find:

- 2 feed samples labeled with the text “additive: OLAQUINDOX” and with a sample code. The samples contain olaquindox in the range between 1 and 15 mg/kg.
- the modified method of analysis (annex 1). By participation you agree with application of this method!
- the reporting form (annex 2). 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; you are asked to use the e-mail address mentioned in the right margin of this letter.
- instructions for handling (milling, storage) of the samples (annex 3).
- a questionnaire (annex 4). 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 samples must be analysed in duplicate.

For recovery purposes we ask you to select a blank piglet feed from your own collection. The reference standard of olaquindox that has to be used (980416) was already sent to you with our letter of 31 May 2000. In the calculations this reference standard can be regarded as 100 % pure.

The deadline for reporting the results is December 14, 2001.

We wish you and your colleagues the best with the collaborative study. 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

cc. mrs. D. Bennink, European Commission, DG Research, Cl/3, Brussels
Annex 1 – Modified method of analysis.

Determination of low level contents of Olaquindox in Feedingstuffs

1. Purpose and scope
   The method is for the determination of olaquindox in feedingstuffs. The limit of determination (=quantification) is 1,5 mg/kg. The limit of detection (=qualification) is 0,1 mg/kg

2. Principle
   The sample is extracted by a water methanol mixture. The content of olaquindox is determined by reversed-phase high-performance liquid chromatography (HPLC) using an UV detector.

3. Reagents

3.1. Methanol
3.2. Methanol, HPLC grade
3.3. Water, HPLC grade
3.4. Mobile phase for HPLC
   Water (3.3)-methanol (3.2) mixture, 900+100 (V + V)
3.5. Standard substance: pure olaquindox 2-[N-2'-(hydroxyethyl)carbamoyl]-3-methylquinoxaline-N1,N4-dioxide, E 851
3.5.1. Olaquindox stock standard solution, 25 μg/ml
   Weigh to the nearest 0,1 mg 5 mg of olaquindox (3.5) in a 200 ml graduated flask and add ca. 190 ml water. Then place the flask for 10 min in an ultrasonic bath (4.1). After ultrasonic treatment, bring the solution to room temperature, make up to the mark with water and mix. Wrap the flask with aluminium foil and store in a refrigerator. At this temperature of ≤ 4°C the solution is stable for 1 month.

3.5.2. Calibration solutions
   Into a series of 50 ml graduated flasks transfer 0.5, 1.0, 2.5, 5.0 and 10.0 ml of the standard stock solution (3.5.1). Make up to the mark with water (3.3) and mix. These solutions correspond to 0.25, 0.5, 1.25, 2.5 and 5.0 μg of olaquindox per ml respectively.

   These solutions must be prepared fresh each day.
4. **Apparatus**

4.1. Ultrasonic bath

4.2. Mechanical shaker

4.3. Membrane filter, 0.45 μm

4.4. HPLC equipment with variable wavelength ultraviolet detector

4.4.1. Liquid chromatographic column, 250 mmx4mm, C18, 5 μm packing, or equivalent.

See remark 7.2.

5. **Procedure**

**Note:** Olaquindox is light sensitive. Carry out all procedures under subdued light or use amber glass ware.

5.1. **General**

5.1.1. Blank feed

For the performance of the recovery test (5.1.2) a blank feed should be analysed to check that neither olaquindox nor interfering substances are present. The blank feed should be similar in type to that of the sample and on analysis olaquindox or interfering substances should not be detected.

5.1.2. Recovery test

A recovery test should be carried out by analysing the blank feed which has been fortified by addition of a quantity of olaquindox, similar to that present in the sample. To fortify at a level of 2.5 mg/kg, transfer 1 ml of the stock standard solution (3.5.1) to a 250 ml conical flask, add 10 g of the blank feed, mix thoroughly and leave for 10 min mixing again several times before proceeding with the extraction step (5.2). In stead of 40 ml water, 39 ml water should be added in the extraction step.

Alternatively, if a blank feed similar in type to that of the sample is not available (see 5.1.1), a recovery test can be performed by means of the standard addition method. In this case, prepare two independent laboratory sample aliquots (A and B) of the feed to be examined. Spike one of them (A), before extraction with a quantity of olaquindox, similar to that already present in the sample. Both samples are analysed. Calculate the analyte content in sample A and B and calculate the recovery by subtraction.

5.2. **Extraction**

Weigh to the nearest 0.01 g, approximately 10 g of the sample. Transfer to a 250 ml conical flask, add 10 ml of methanol (3.1) and place the flask for 5 min in the ultra-
sonic bath (4.1). Add 40 ml water and leave in the ultrasonic bath for further 15 min. Remove the flask from the ultrasonic bath, shake it for 30 min on the shaker (4.2) and filter through a folded filter or a glass fibre filter (GFA, Whatman) (see remark 7.1). It is highly recommended to filter the clear samples by using a membrane filter (4.3) additionally. Proceed to the HPLC determination (5.3).

5.3. HPLC determination

5.3.1. Parameters:
The following conditions are offered for guidance, other conditions may be used provided that they give equivalent results.
Analytical column (4.4.1). See remark 7.2.
Mobile Phase (3.4): water (3.3) - methanol (3.1.) mixture, 900 + 100 (V+ V)
Flow rate: 1.5 - 2 ml/min
Detection wavelength: 380 nm
Injection volume: 50 µl -100 µl
Check the stability of the chromatographic system, injecting several times the calibration solution (3.5.3) containing 1.25 µg/ml, until constant peak heights and retention times are achieved.

5.3.2. Calibration graph
Inject each calibration solution (3.5.3) several times and determine the mean peak heights (areas) for each concentration. Plot a calibration graph using the mean peak heights (areas) of the calibration solutions as the ordinates and the corresponding concentrations in pg/ml as the abscissae.

5.3.3. Sample solution
Inject the sample extract (5.2) and determine the peak height (area) of the olaquindox peaks.

6. Calculation of the results
From the height (area) of the olaquindox peaks of the sample solution determine the concentration of the sample solution in µg/ml by reference to the calibration graph (5.3.2).
The olaquindox content w (mg/kg) of the sample is given by the following formular:

\[ w = \frac{c \times 50}{m} \]
in which:

\[ c = \text{olaquindox concentration of the sample extract (5.2) in } \mu\text{g/ml} \]
\[ m = \text{mass of the test portion in g} \]

7 **Remarks**

7.1 Instead of filtration through a folded filter a centrifugation step could be carried out. If plastic vials are used for centrifugation, a recovery study should be carried out to validate this application.

7.2 The following columns could be recommended: Hypersil ODS 5 \( \mu \)m 200 x 4.6 mm, Spherisorb ODS 2 5 \( \mu \)m 250 x 4.6 mm, LUNA C18(2) 5 \( \mu \)m 250 x 4.6 mm.
CANFAS COLLABORATIVE STUDIES - 2ND ROUND - NOVEMBER 2001

ANNEX 2 - Report form

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: Task 4 COLLABORATIVE STUDY - 2nd round
Contact person: e-mail: 
fax: 
telephone: 

Date of analysis: 

Analyte: OLAQUINDOX

<table>
<thead>
<tr>
<th>Unit Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
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<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Annex 3 - Instructions for handling of the samples

1. Storage
   Store the samples at room temperature until analysis. Protect the material from direct light.

2. Milling
   Grind the feed samples with a mill equipped with a 1 mm screen

3. Mixing of the test samples before weighing
   Mix the entire sample thoroughly
ADDITIVE: OLAQUINDOX

Annex 4 - Questionnaire

Laboratory: .................................................................................................................................
Contact person: .............................................................................................................................

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

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 olaquindox: ....... min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample (from your own collection and to be used for recovery purposes)

Please indicate the olaquindox peak with an arrow

Recovery results:

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

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 co-operation!
APPENDIX 2

Composition of the feed samples
2 250.00 Biggen opfok korrel
biggenvoer van 12 tot 25/30 kg

Grondstof | Silo Gewicht Tol. Cumul Gew. Charge Charge
<table>
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<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
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<tr>
<td></td>
<td>kg +/-Afw. kg</td>
<td>24</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

Weegschaal DW 1

<table>
<thead>
<tr>
<th>Grondstof</th>
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<th>Tol. Gewicht</th>
<th>Cumul Gewicht</th>
</tr>
</thead>
<tbody>
<tr>
<td>113 Zonbl.schr.290re</td>
<td>(2) 2.00</td>
<td>0.30</td>
<td>10.00</td>
</tr>
<tr>
<td>460 Tapiocca65%zetmeel</td>
<td>(4) 7.50</td>
<td>1.13</td>
<td>47.50</td>
</tr>
<tr>
<td>77 Soja 45/46(arg/braz)</td>
<td>(9) 13.00</td>
<td>1.95</td>
<td>112.50</td>
</tr>
</tbody>
</table>

Weegschaal DW 2

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<th>Tol. Gewicht</th>
<th>Cumul Gewicht</th>
</tr>
</thead>
<tbody>
<tr>
<td>145 Tarwe (voer)</td>
<td>(9) 10.00</td>
<td>1.50</td>
<td>50.00</td>
</tr>
<tr>
<td>14 Gerst</td>
<td>(11) 37.10</td>
<td>5.57</td>
<td>235.50</td>
</tr>
<tr>
<td>40 Mais</td>
<td>(12) 12.00</td>
<td>1.80</td>
<td>295.50</td>
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Bijstort SP4

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<th>Cumul Gewicht</th>
</tr>
</thead>
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<tr>
<td>34 Lynzaad</td>
<td>(0) 5.00</td>
<td>0.75</td>
<td>25.00</td>
</tr>
<tr>
<td>105 Vismeel 65.9% re</td>
<td>(0) 4.40</td>
<td>0.66</td>
<td>47.00</td>
</tr>
</tbody>
</table>

Bijstort SP6

<table>
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<th>Cumul Gewicht</th>
</tr>
</thead>
<tbody>
<tr>
<td>476 Powerfood Twil melkv</td>
<td>(0) 4.00</td>
<td>0.60</td>
<td>20.00</td>
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Bijstort SP7

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<th>Cumul Gewicht</th>
</tr>
</thead>
<tbody>
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<td>21 Fumaarzuur</td>
<td>(0) 0.25</td>
<td>0.01</td>
<td>1.25</td>
</tr>
<tr>
<td>78 L-lysine HCl</td>
<td>(0) 0.17</td>
<td>0.01</td>
<td>2.10</td>
</tr>
<tr>
<td>79 DL-Methio-nine</td>
<td>(0) 0.03</td>
<td>0.00</td>
<td>2.25</td>
</tr>
<tr>
<td>117 Krijt/kalksteen</td>
<td>(0) 0.45</td>
<td>0.02</td>
<td>4.50</td>
</tr>
<tr>
<td>228 Monocal Belgie</td>
<td>(0) 0.50</td>
<td>0.03</td>
<td>7.00</td>
</tr>
<tr>
<td>485 Zout</td>
<td>(0) 0.10</td>
<td>0.01</td>
<td>7.50</td>
</tr>
<tr>
<td>508 Prem biggen Rikilt</td>
<td>(0) 1.00</td>
<td>0.05</td>
<td>12.50</td>
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</table>

Vloeistoffen

<table>
<thead>
<tr>
<th>Vloeistof</th>
<th>Silo Gewicht</th>
<th>Tol. Gewicht</th>
<th>Cumul Gewicht</th>
</tr>
</thead>
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<tr>
<td>474 Melasse riet &gt;450s</td>
<td>(3) 2.50</td>
<td>0.38</td>
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</tr>
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</table>

Totaal: 500.00

---

RETOURPRODUKT

---

INSTELLINGEN

<table>
<thead>
<tr>
<th>T.R.</th>
<th>%</th>
<th>50%</th>
</tr>
</thead>
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<tr>
<td>V.Z.</td>
<td>%</td>
<td>80%</td>
</tr>
<tr>
<td>Z.F.</td>
<td>2%</td>
<td>mm</td>
</tr>
<tr>
<td>H.M.</td>
<td>hood/laag toeren kringloop</td>
<td>Ja/Nee</td>
</tr>
<tr>
<td>L.M.</td>
<td>voormengen</td>
<td>60. sec</td>
</tr>
<tr>
<td>M.D.</td>
<td>%</td>
<td>1/h</td>
</tr>
<tr>
<td>Meel temp</td>
<td>°C</td>
<td>korrels 77°C</td>
</tr>
<tr>
<td>Matrijs diam.</td>
<td>25 x 35. mm</td>
<td></td>
</tr>
<tr>
<td>K.P.</td>
<td>Amp</td>
<td></td>
</tr>
<tr>
<td>Laagdikte Ko</td>
<td>cm</td>
<td></td>
</tr>
<tr>
<td>Zeef Ko</td>
<td>mm</td>
<td></td>
</tr>
<tr>
<td>Kruimelen</td>
<td>ja/nee</td>
<td></td>
</tr>
<tr>
<td>Eindvochtgehalte</td>
<td>12.0%</td>
<td></td>
</tr>
<tr>
<td>Cons.</td>
<td>%</td>
<td>65/35</td>
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</table>
### BESTMIX - Afdruk kostenformulier

2 250.00 Biggen opfok korrel
biggenvoer van 12 tot 25/30 kg

<table>
<thead>
<tr>
<th>Nr</th>
<th>Grondstofnaam</th>
<th>Aandeel</th>
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<td>14</td>
<td>Gerst</td>
<td>37.10000</td>
<td>185.500</td>
</tr>
<tr>
<td>77</td>
<td>Soja 45/46(arg/braz)</td>
<td>13.00000</td>
<td>65.000</td>
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<td>40</td>
<td>Mais</td>
<td>12.00000</td>
<td>60.000</td>
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<tr>
<td>145</td>
<td>Tarwe (voer)</td>
<td>10.00000</td>
<td>50.000</td>
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<tr>
<td>460</td>
<td>Tapioca65%zetmeel</td>
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<td>37.500</td>
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<tr>
<td>34</td>
<td>Lyndaad</td>
<td>5.00000</td>
<td>25.000</td>
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<tr>
<td>105</td>
<td>Vismeel 65.9% re</td>
<td>4.40000</td>
<td>22.000</td>
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<td>476</td>
<td>Powerfood Twil melkv</td>
<td>4.00000</td>
<td>20.000</td>
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<td>Melasse riet &gt;450s</td>
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<td>10.000</td>
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<td>Prem biggen Rikilt</td>
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<td>5.000</td>
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<td>Monocal Belgie</td>
<td>0.50000</td>
<td>2.500</td>
</tr>
<tr>
<td>117</td>
<td>Krijt/kalksteen</td>
<td>0.45000</td>
<td>2.250</td>
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<td>78</td>
<td>L-lysine HCl</td>
<td>0.17000</td>
<td>0.850</td>
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<td>485</td>
<td>Zout</td>
<td>0.10000</td>
<td>0.500</td>
</tr>
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<td>79</td>
<td>DL-Methio-nine</td>
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<td>0.150</td>
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</tbody>
</table>

| Totaal 100.00000 | 500.000kg |

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<th>Nr</th>
<th>Nutrient</th>
<th>Berekend</th>
<th>Verschil</th>
<th>Minimum</th>
<th>Maximum</th>
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<td>1</td>
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<td>181.11 g</td>
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<td>180.00</td>
<td>200.00</td>
</tr>
<tr>
<td>2</td>
<td>Rvet</td>
<td>42.70 g</td>
<td>*</td>
<td>45.00</td>
<td>*</td>
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<tr>
<td>3</td>
<td>Rc</td>
<td>43.83 g</td>
<td>*</td>
<td>*</td>
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<tr>
<td>4</td>
<td>Vocht</td>
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<td>*</td>
<td>*</td>
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<tr>
<td>5</td>
<td>Ras</td>
<td>58.90 g</td>
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<td>*</td>
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<tr>
<td>6</td>
<td>Zetmeel</td>
<td>393.49 g</td>
<td>-26.51 g</td>
<td>420.00</td>
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<tr>
<td>8</td>
<td>Ca</td>
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<td>7.00</td>
<td>9.00</td>
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<tr>
<td>9</td>
<td>P</td>
<td>6.36 g</td>
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<td>1.00</td>
<td>*</td>
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<tr>
<td>11</td>
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<td>11.37 g</td>
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<td>*</td>
<td>*</td>
</tr>
<tr>
<td>12</td>
<td>Methion</td>
<td>3.84 g</td>
<td>*</td>
<td>*</td>
<td>*</td>
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<tr>
<td>13</td>
<td>Meth+cys</td>
<td>6.97 g</td>
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<td>*</td>
<td>*</td>
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<tr>
<td>14</td>
<td>Trypt.</td>
<td>2.21 g</td>
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<td>*</td>
<td>*</td>
</tr>
<tr>
<td>15</td>
<td>Threon.</td>
<td>6.81 g</td>
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<td>*</td>
<td>*</td>
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<tr>
<td>16</td>
<td>Isoleuc</td>
<td>6.78 g</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>19</td>
<td>Linolz.</td>
<td>10.07 g</td>
<td>*</td>
<td>10.00</td>
<td>*</td>
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<tr>
<td>30</td>
<td>EW*100</td>
<td>106.96 g</td>
<td>-0.04 g</td>
<td>107.00</td>
<td>107.00</td>
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<tr>
<td>32</td>
<td>P-vert</td>
<td>3.58 g</td>
<td>*</td>
<td>3.00</td>
<td>*</td>
</tr>
<tr>
<td>34</td>
<td>dvLys v</td>
<td>9.53 g</td>
<td>*</td>
<td>9.00</td>
<td>*</td>
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<tr>
<td>35</td>
<td>dvmet</td>
<td>3.19 g</td>
<td>*</td>
<td>3.00</td>
<td>*</td>
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<td>36</td>
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<td>5.40</td>
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<td>dvtryp v</td>
<td>1.72 g</td>
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<td>1.70</td>
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<td>-0.09 g</td>
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<tr>
<td>50</td>
<td>Cu</td>
<td>73.96 mg</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>51</td>
<td>Na</td>
<td>1.78 g</td>
<td>*</td>
<td>1.50</td>
<td>3.00</td>
</tr>
<tr>
<td>53</td>
<td>K</td>
<td>8.74 g</td>
<td>*</td>
<td>12.00</td>
<td>*</td>
</tr>
<tr>
<td>54</td>
<td>Cl</td>
<td>3.98 g</td>
<td>*</td>
<td>1.50</td>
<td>*</td>
</tr>
<tr>
<td>59</td>
<td>Gewicht</td>
<td>100.00 g</td>
<td>*</td>
<td>100.00</td>
<td>100.00</td>
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<tr>
<td>100</td>
<td>vit. A</td>
<td>4000.00 i.e.</td>
<td>*</td>
<td>*</td>
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<tr>
<td>101</td>
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<td>*</td>
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<td>102</td>
<td>vit.E</td>
<td>734.68 mg</td>
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<td>*</td>
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<tr>
<td>103</td>
<td>BHA/ethyl</td>
<td>0.02 mg</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>106</td>
<td>ethopab</td>
<td>13.00 mg</td>
<td>*</td>
<td>*</td>
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</tr>
</tbody>
</table>
APPENDIX 3

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

Homogeneity test 2\textsuperscript{nd} collaborative study

Additive: Olaquindox
Product: Piglet feed, 10 ppm

Date of determination: October 29\textsuperscript{th}, 2001

<table>
<thead>
<tr>
<th>Sample</th>
<th>Content ppm</th>
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<tbody>
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<td>10,5</td>
</tr>
<tr>
<td>345314</td>
<td>10,2</td>
</tr>
<tr>
<td>345327</td>
<td>10,6</td>
</tr>
<tr>
<td>345328</td>
<td>10,3</td>
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<td>345344</td>
<td>8,7</td>
</tr>
<tr>
<td>345345</td>
<td>8,7</td>
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<td>345363</td>
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<td>345393</td>
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<tr>
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<td>10,4</td>
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Homogeneity

Criterion: $\text{CV}_{\text{between}} = < 15\%$

<table>
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<th>OK</th>
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<tbody>
<tr>
<td>Average (ppm)</td>
<td>9,90</td>
</tr>
<tr>
<td>SD (between samples)</td>
<td>0,940</td>
</tr>
<tr>
<td>CV (between samples)</td>
<td>9,5</td>
</tr>
<tr>
<td>Grubb's test, single lower</td>
<td>1,696</td>
</tr>
<tr>
<td>Grubb's test, single upper</td>
<td>1,016</td>
</tr>
<tr>
<td>Grubb's test, double lower</td>
<td>0,5574</td>
</tr>
<tr>
<td>Grubb's test, double upper</td>
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</tbody>
</table>
### Homogeneity test 2\textsuperscript{nd} collaborative study

**Additive:** Olaquindox  
**Product:** Piglet feed, 2.5 ppm  
**Date of determination:** October 29\textsuperscript{th}, 2001

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<td>345334</td>
<td>3.2</td>
</tr>
<tr>
<td>345339</td>
<td>3.1</td>
</tr>
<tr>
<td>345371</td>
<td>3.1</td>
</tr>
<tr>
<td>345392</td>
<td>2.6</td>
</tr>
<tr>
<td>346402</td>
<td>3.2</td>
</tr>
<tr>
<td>345412</td>
<td>2.9</td>
</tr>
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<td>345432</td>
<td>2.9</td>
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</table>

#### Homogeneity criterion: $CV_{between} = < 15\%$

<table>
<thead>
<tr>
<th>Average (ppm)</th>
<th>2.90</th>
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</thead>
<tbody>
<tr>
<td>SD (between samples)</td>
<td>0.219</td>
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<td>CV (between samples)</td>
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<td>Grubb's test, single lower</td>
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<tr>
<td>Grubb's test, single upper</td>
<td>1.372 no outlier</td>
</tr>
<tr>
<td>Grubb's test, double lower</td>
<td>0.5574 no outliers</td>
</tr>
<tr>
<td>Grubb's test, double upper</td>
<td>0.5574 no outliers</td>
</tr>
</tbody>
</table>

Result of Grubb's test: no outliers
APPENDIX 4

Sample codes
Sample codes supplied to the participants in the olaquindox collaborative study, 2nd round

<table>
<thead>
<tr>
<th>Participant code</th>
<th>OLA2 piglet 2.5 ppm OLA 1a</th>
<th>OLA2 piglet 2.5 ppm OLA 1b</th>
<th>OLA2 piglet 10 ppm OLA 2a</th>
<th>OLA2 piglet 10 ppm OLA 2b</th>
</tr>
</thead>
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</table>
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms
of partner 12
## CANFAS

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

### Task 4 COLLABORATIVE STUDY - 2nd round

### Analyte: OLAQUINDOX

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
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<td>6,99</td>
</tr>
<tr>
<td>125397</td>
<td>1,90</td>
<td>2,29</td>
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<tr>
<td>125419</td>
<td>1,71</td>
<td>1,34</td>
</tr>
<tr>
<td>125421</td>
<td>6,57</td>
<td>6,74</td>
</tr>
</tbody>
</table>

**Subtitle:**

**Lab-name:**

**Contact person:**

**Date of analysis:** 14-01-2002

**e-mail:**

**fax:**

**telephone:**
Date(s) of analysis: 21-dec-01

Chromatographic conditions:

Column:
1- As described in the method:
2- Other: Tracer extrasil ODS 5x25x0,46

Mobile phase
1- As described in the method: yes
2- Other:

Flow rate: 1,3 ml/min-1
Injection volume: 150 ul
Retention time of olaquindox: 9,7 min.

Cromatograms: In the file word annex!

Recovery results:
1- Percentage recovery: 94,7% and 91,4%
2- Single / duplicate determinations: no
3- If duplicate, please give both percentages:
4- Spiking level: 25 and 50 ug
Signal 1: DAD1 A, Sig=380,4 Ref=500,80

<table>
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<tr>
<th>RetTime [min]</th>
<th>Type</th>
<th>Area [mAU*s]</th>
<th>Amount [ug/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.752 FB</td>
<td>189.33951</td>
<td>1.775131e-3</td>
<td>3.31593e-1</td>
</tr>
</tbody>
</table>

Grp Name: Olaquindox

Totals: 3.31593e-1

Results obtained with enhanced integrator!

======================================
**End of Report***
<table>
<thead>
<tr>
<th>RetTime</th>
<th>Type</th>
<th>Area [mAU*s]</th>
<th>Amt/Area [ug/ml]</th>
<th>Amount [ug/ml]</th>
<th>Grp</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.577</td>
<td>PB</td>
<td>197.40469</td>
<td>1.75131e-3</td>
<td>3.45718e-1</td>
<td></td>
<td>Olaquindox</td>
</tr>
</tbody>
</table>

Totals:  

Results obtained with enhanced integrator!
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms

of partner 15
CANFAS COLLABORATIVE STUDIES - 2ND ROUND - NOVEMBER 2001

ANNEX 2 - Report form

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 - 2nd round
Lab-name: 
Contact person: 
e-mail: 
fax: 
telephone: 
Date of analysis: 30,11,2001
Analyte: OLAQUINDOX

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>155316</td>
<td>10,68</td>
<td>10,41</td>
</tr>
<tr>
<td>155332</td>
<td>2,85</td>
<td>2,63</td>
</tr>
<tr>
<td>155400</td>
<td>9,91</td>
<td>9,50</td>
</tr>
<tr>
<td>155407</td>
<td>2,89</td>
<td>2,92</td>
</tr>
</tbody>
</table>
CANFAS COLLABORATIVE STUDIES – 2nd round – NOVEMBER 2001

ADDITIVE: OLAQUINDOX

Annex 4 - Questionnaire

Laboratory: ..
Contact person: ..

Date(s) of analysis: ...

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ☑ Other: JUCERIL ODS-3, 5 µm x 350 mm x 1.6 mm

- Mobile phase:
  - ☑ As described in the method
  - ☐ Other: ...

- Flow-rate: ...

- Injection volume: ...

- Retention time of olaquindox: ...

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample (from your own collection and to be used for recovery purposes)

Please indicate the olaquindox peak with an arrow

Recovery results:

- Percentage recovery: ...
- Single / duplicate determinations: ☑ single ☐ duplicate
- If duplicate, please give both percentages: 88.8% and 92.8%
- Spiking level: ...

mg/kg
External Standard Report

By: Signal

Data Modified: Monday, December 03, 2001 10:07:00 AM

n: 1.0000

1: DAD1 A, Sig=380.4 Ref=450.50

<table>
<thead>
<tr>
<th>Type</th>
<th>Area</th>
<th>Amt/Area</th>
<th>Amount</th>
<th>Grp</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[mAU's]</td>
<td>[ng/ul]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 BB</td>
<td>88.08059</td>
<td>2.91140e-2</td>
<td>9.91049</td>
<td>olaquindox</td>
<td>9.91049</td>
</tr>
</tbody>
</table>

s obtained with enhanced integrator!

*** End of Report ***
**External Standard Report**

**Data Modified:** Monday, December 03, 2001 10:07:00 AM

**Type Area Amount Grp Name**

<table>
<thead>
<tr>
<th>[mAU*s]</th>
<th>[ng/ul]</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.10433</td>
<td>2.87803e-2</td>
</tr>
</tbody>
</table>

**Results obtained with enhanced integrator**

***End of Report***
## External Standard Report

**By:** Signal

**Data Modified:** Monday, December 03, 2001 10:07:00 AM

**Data:**

<table>
<thead>
<tr>
<th>Type</th>
<th>Area</th>
<th>Amount/Area</th>
<th>Amount</th>
<th>Grp</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Data:**

1. **DAD1 A, Sig=380,4 Ref=450,50**

**Measurements:**

- Area: 0.0000

**Remarks:**

- Obtained with enhanced integrator.
- No errors or corrections.

**Note:** Calibrated compound(s) not found.
Spike = 2.5 ng/kg

---

**External Standard Report**

<table>
<thead>
<tr>
<th>Type</th>
<th>Area</th>
<th>Amt/Area</th>
<th>Amount</th>
<th>Grp</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(mAU*s)</td>
<td>(ng/ul)</td>
<td></td>
<td>olaquindox</td>
</tr>
<tr>
<td>BB</td>
<td>16.21838</td>
<td>2.6401e-2</td>
<td>2.32248</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- Retention time: 10.809s
- Obtained with enhanced integrator

**Equation:**
\[ \text{REC} = \frac{2.32}{2.5} \times 100 \approx 92.8\% \]

---

**End of Report***
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 16
CANFAS COLLABORATIVE STUDIES - 2ND ROUND - NOVEMBER 2001

ANNEX 2 - Report form

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 - 2nd round
Lab-name: 
Contact person: 
e-mail: 
fax: 
telephone: 

Date of analysis: Nov. 20/21, 2001

Analyte: OLAQUINDOX

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>165389</td>
<td>9,73</td>
<td>9,52</td>
</tr>
<tr>
<td>165403</td>
<td>2,70</td>
<td>2,63</td>
</tr>
<tr>
<td>165423</td>
<td>2,60</td>
<td>2,71</td>
</tr>
<tr>
<td>165427</td>
<td>9,65</td>
<td>9,49</td>
</tr>
</tbody>
</table>
CANSAS COLLABORATIVE STUDIES - 2nd round - NOVEMBER 2001

ADDITIVE: OLAQUINDOX

Annex 4 - Questionnaire

Laboratory: 
Contact person: 

Date(s) of analysis: November 20./21., 2001

Chromatographic conditions:
- Column:
  - □ As described in the method
  - x Other: Phenomenex LUNA C18(2), 5 µm, 150 x 4.6 mm
- Mobile phase:
  - x As described in the method
  - □ Other:

- Flow-rate: 1.3 ml/min
- Injection volume: 20 µl
- Retention time of olaquindox: 6.9 min

Chromatograms: Please include representative chromatograms of:
- Blind positive feed samples
- Blind blank feed sample

Please indicate the olaquindox peak with an arrow

Recovery results:
- Percentage recovery: 74.7 %
- Single / duplicate determinations: □ single x duplicate
- If duplicate, please give both percentages: 75.3 and 74.0 %
- Spiking level: 3.65 mg/kg
### FILE: 3 CALC-METHOD: EXT-STD TABLE: 9 CONC: HEIGHT

<table>
<thead>
<tr>
<th>NO.</th>
<th>RT</th>
<th>AREA</th>
<th>HEIGHT</th>
<th>UG/ML</th>
<th>NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.28</td>
<td>101796</td>
<td>5552</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.58</td>
<td>20992</td>
<td>263</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>122788</td>
<td>5815</td>
<td>0.006</td>
<td></td>
</tr>
</tbody>
</table>

**PEAK REJ:** 100
**SF:** 1.000
**SAMP-AMT:** 1.000

D-2500

11/20/01 02:29
INJ NO. OF STD : 1 / 1 REP , 1st level

FILE: 3 CALC-METHOD: EXT-STD TABLE: 8 CONC: AREA

<table>
<thead>
<tr>
<th>NO.</th>
<th>RT</th>
<th>AREA</th>
<th>HEIGHT</th>
<th>UG/ML</th>
<th>NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6.92</td>
<td>21683</td>
<td>1380</td>
<td>0.730</td>
<td>OLAQUI</td>
</tr>
</tbody>
</table>

Sample code 16542

FILE: 3 CALC-METHOD: EXT-STD TABLE: 8 CONC: AREA

<table>
<thead>
<tr>
<th>NO.</th>
<th>RT</th>
<th>AREA</th>
<th>HEIGHT</th>
<th>UG/ML</th>
<th>NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.16</td>
<td>466313</td>
<td>35283</td>
<td>0.466</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.84</td>
<td>5756</td>
<td>763</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.10</td>
<td>1303</td>
<td>34</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3.44</td>
<td>1792</td>
<td>119</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.90</td>
<td>1525</td>
<td>64</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4.52</td>
<td>695</td>
<td>56</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6.90</td>
<td>37303</td>
<td>3813</td>
<td>1.973</td>
<td>OLAQUI</td>
</tr>
</tbody>
</table>
INJ NO. OF STD: 1 / 1 REP, 1st level

FILE: 3 CALC-METHOD: EXT-STD TABLE: 8 CONC: AREA

<table>
<thead>
<tr>
<th>NO.</th>
<th>RT</th>
<th>AREA</th>
<th>HEIGHT</th>
<th>UG/ML</th>
<th>NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6.88</td>
<td>21662</td>
<td>1455</td>
<td>0.730</td>
<td>Olaquindor</td>
</tr>
</tbody>
</table>
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 17
CANFAS COLLABORATIVE STUDIES - 2ND ROUND - NOVEMBER 2001

ANNEX 2 - Report form

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 - 2nd round
Lab-name: __________________________
Contact person: __________________________
e-mail: __________________________
fax: __________________________
telephone: __________________________

Date of analysis: 9.10.01.2002

Analyte: OLAQUINDOX

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>175313</td>
<td>2,97</td>
<td>3,03</td>
</tr>
<tr>
<td>175331</td>
<td>10,30</td>
<td>10,60</td>
</tr>
<tr>
<td>175352</td>
<td>10,05</td>
<td>9,97</td>
</tr>
<tr>
<td>175431</td>
<td>3,00</td>
<td>2,95</td>
</tr>
</tbody>
</table>
CANFAS COLLABORATIVE STUDIES – 2nd round - NOVEMBER 2001

ADDITIVE: OLAQUINDOX

Annex 4 - Questionnaire

Laboratory: ..
Contact person: ..

Date(s) of analysis: 9. m. 10. 1. 2002

Chromatographic conditions:
- Column:
  - As described in the method
  - Other: Spherisorb, S10, ODS-1
- Mobile phase:
  - As described in the method
  - Other: ...
- Flow rate: 1.0 ml/min
- Injection volume: 20 µl
- Retention time of olaquindox: 6.3 min

Chromatograms: Please include representative chromatograms of:
- Blind positive feed samples
- Blind blank feed sample (from your own collection and to be used for recovery purposes)

Please indicate the olaquindox peak with an arrow

Recovery results:
- Percentage recovery: 88%
- Single/duplicate determinations: single
- If duplicate, please give both percentages: ... % and ... %
- Spiking level: 3.75 mg/kg

Percentage recovery: 88% single
Spiking level: 10 mg/kg
**D-7000 HSM: Olaquindox**

**Series:** 0226

**Sample Name:** 175313

**Analyzed:** 09.01.02 13:58

**Data Path:** C:\Win32App\HSM\OLAQU\DATA\0226\  
**Application:** Olaquindox

**Injection from this vial:** 1 of 1

**Sample Description:**

Chrom Type: Fixed WL Chromatogram, 380 nm

### Acquisition Method: Olaquindox
- **Column Type:** RP 18
- **Pump A Type:** L-7100
- **Solvent A:** MeOH/H2O  
- **Solvent C:** MeOH/H2O
- **Solvent B:** MeOH/H2O  
- **Solvent D:** MeOH/H2O
- **Peak Quantitation:** AREA
- **Calculation Method:** EXT-STD

### Peak Table

<table>
<thead>
<tr>
<th>Name</th>
<th>RT</th>
<th>Area</th>
<th>Conc 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olaqu</td>
<td>4,28</td>
<td>431</td>
<td>0,000</td>
</tr>
<tr>
<td></td>
<td>6,89</td>
<td>7772</td>
<td>2,973</td>
</tr>
</tbody>
</table>

**Sample Amount:** 0,200

**Scale Factor 1:** 1,000

**Peak rejection level:** 0
Sample Name: 175352

Analyzed: 10.01.02 11:58
Reported: 15.01.02 10:20
Processed: 15.01.02 10:19

Data Path: C: \ Win32App \ HSM \ OLAQU \ DATA \ 0229 \ Series: 0229
Application: Olaquindox
Injection from this vial: 1 of 1
Vial Number: 4
Volume: 20,0 ul

Sample Description:

Chrom Type: Fixed WL Chromatogram, 380 nm

```
<table>
<thead>
<tr>
<th>Name</th>
<th>RT</th>
<th>Area</th>
<th>Conc 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olaqu</td>
<td>6,19</td>
<td>0</td>
<td>0,000</td>
</tr>
<tr>
<td></td>
<td>6,88</td>
<td>29814</td>
<td>9,967</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>29814</td>
</tr>
</tbody>
</table>
```

Peak rejection level: 0
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 18
<table>
<thead>
<tr>
<th>Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>185350</td>
<td>2.10</td>
<td>1.90</td>
</tr>
<tr>
<td>185353</td>
<td>2.10</td>
<td>2.50</td>
</tr>
<tr>
<td>185368</td>
<td>8.60</td>
<td>8.50</td>
</tr>
<tr>
<td>185395</td>
<td>9.40</td>
<td>9.60</td>
</tr>
</tbody>
</table>
CANFAS COLLABORATIVE STUDIES - 2nd round - NOVEMBER 2001

ADDITIVE: OLAQUINDOX

Annex 4 - Questionnaire

Laboratory: ..........................................................
Contact person: ..........................................................

Date(s) of analysis: ............................................. 10/12/2001

Chromatographic conditions:

- Column:
  - As described in the method: Spherisorb ODS2; 250 x 4.6 mm; 5 µm
  - Other: ..........................................................

- Mobile phase:
  - As described in the method: ..........................................................
  - Other: ..........................................................

- Flow-rate: ....... ml/min
- Injection volume: ....50... µl
- Retention time of olaquindox: ..., min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample (from your own collection and to be used for recovery purposes)

Please indicate the olaquindox peak with an arrow

Recovery results:

- Percentage recovery: ....7..%
- Single / duplicate determinations: □ single  X duplicate
- If duplicate, please give both percentages: 62. % and 72. %
- Spiking level: ....5.... mg/kg
Current Chromatogram(s)

DAD1 A, Sig=380.4 Ref=550,100 (ADD1210\CANFAS22.D)
DAD1 A, Sig=380.4 Ref=550,100 (ADD1210\CANFAS23.D)

Blanc N° 185823

Blanc N° 185823 spiked 2.5 mg/Kg

Of window 38: Current...
Current Chromatogram(s)

DAD1 A, Sig=380.4 Ref=550,100 (AD011210/CANFAS28.D)

N° 185360 10 g/ 50 ml

Area 18.7502
Current Chromatogram(s)

DAD1 A, Sig=380.4 Ref=550,100 (AD011210\CANFA532 D)

N° 185368 10 g/50 ml

Ansi. 224.056
Target + Library Spectrum

\*DAD1, 8.359 (1.4 mAU, \text{-}) \text{Ref=8.146 & 9.185 of CANFAS28.D}

\*Olaquindox

- N° 186360 10 g/50 ml
- Olaquindox standard 1.25 \text{μg/ml}

match = 858.7

![Graph showing the spectrum of Olaquindox with specified conditions and matches.](image)
Target + Library Spectrum

*DAD1, 8.465 (9.3 mAU, -) Ref=7.986 & 9.239 of CANFAS32.D

*Olaquindox

N° 185368 10 g/50 ml

Olaquindox standard 1.25 µg/ml

match = 986
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms

of partner 20
CANFAS COLLABORATIVE STUDIES - 2ND ROUND - NOVEMBER 2001

ANNEX 2 - Report form

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 - 2nd round
Lab-name: 
Contact person: e-mail: 
fax: 
telephone: 
Date of analysis: 24/11-04/12/2001
Analyte: OLAQUINDOX

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>205367</td>
<td>9,09</td>
<td>9,10</td>
</tr>
<tr>
<td>205377</td>
<td>2,31</td>
<td>2,33</td>
</tr>
<tr>
<td>205417</td>
<td>9,00</td>
<td>8,99</td>
</tr>
<tr>
<td>205428</td>
<td>2,44</td>
<td>2,38</td>
</tr>
</tbody>
</table>
Laboratory: 
Contact person: 

Date of analysis: December 2001

Chromatographic conditions:
- Column:
  - ☐ As described in the method
  - ✔ Other: ODS Hypersyl C18 200x4.6 mm, 5 µm
- Mobile phase:
  - ✔ As described in the method
  - ☐ Other
- Flow rate: 1.5 ml/min
- Injection volume: 100 µl
- Retention time of nicarbazin: 6.8 min

Chromatograms: Please include representative chromatograms of:
- Blind positive feed sample
- Blind blank feed sample

Recovery results:
- Percentage recovery: 87.7 %
- Duplicate determination: 87.8 % and 87.6 %
- Spiking level: 2.5 mg/Kg
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms
of partner 21
Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

Subtitle: OLAQUINDOX

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>215341</td>
<td>3,40</td>
<td>3,43</td>
</tr>
<tr>
<td>215351</td>
<td>9,97</td>
<td>9,41</td>
</tr>
<tr>
<td>215401</td>
<td>3,35</td>
<td>3,48</td>
</tr>
<tr>
<td>215422</td>
<td>9,80</td>
<td>9,16</td>
</tr>
</tbody>
</table>
CANFAS COLLABORATIVE STUDIES – 2nd round - NOVEMBER 2001

ADDITIVE: OLAQUINDOX

Annex 4 - Questionnaire

Laboratory: ........................................
Contact person: ...........................................................

Date(s) of analysis: 22/11 e 23/11/2001

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ☑ Other: SUPERCOIL...CC 18...25 cm x 4,6 mm + SUPERGUARD
  - ☑ Other: GRADIENT ELUTION (ACETONITRILE - ACETATE BUFFER 90:10 pH 4,6)
- Mobile phase:
  - ☐ As described in the method
  - ☑ Other: ...
- Flow-rate: ........................................... ml/min
- Injection volume: 50 µl
- Retention time of olaquindox: 7,65 min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample (from your own collection and to be used for recovery purposes)

Please indicate the olaquindox peak with an arrow

Recovery results:

- Percentage recovery: 99,2%
- Single / duplicate determinations: ☑ single ☐ duplicate
- If duplicate, please give both percentages: 98,2% and 99,2%
- Spiking level: 2,5... mg/kg
Data File

Injection Date: 22/11/2001 14.27.01
Sample Name: 5422-A
Acq. Operator:
Analysis Method: C:\H2
Last changed: 23/11/2001 9.43.43
(modified after loading)

DAD1 A, Sig=365,8 Ref=off

External Standard Report

Sorted By: Signal
Multiplier: 1.0000
Dilution: 1.0000

Signal 1: DAD1 A, Sig=365,8 Ref=off

<table>
<thead>
<tr>
<th>RetTime</th>
<th>Type</th>
<th>Area</th>
<th>Amt/Area</th>
<th>Amount</th>
<th>Grp</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.659</td>
<td>MM</td>
<td>215.14258</td>
<td>4.55740e-1</td>
<td>98.04917</td>
<td>OLAQ-olaquindox</td>
<td></td>
</tr>
</tbody>
</table>

Totals: 98.04917

Results obtained with enhanced integrator!
Injection Date: 22/11/2001 16.48.06
Sample Name: SPURED D SAMPLE
Acq. Operator: 
Last changed: 23/11/2001 10.38.09
Analysis Method: (modified after loading)

External Standard Report

Sorted By: Signal
Multiplier: 1.0000
Dilution: 1.0000

Signal 1: DAD1 A, Sig=365.8 Ref=off
Ret Time Type | Area [mAU*s] | Amt/Area [ng inj] | Amount Grp Name
--- | --- | --- | ---
7.643 MM | 56.26999 | 4.47699e-1 | 25.19200 OLAQ-olaquindox

Totals: 25.19200

Results obtained with enhanced integrator!
Injection Date : 22/11/2001 16.24.33
Sample Name : 
Acq. Operator : BLANKFEED
Acq. Method : 
Last changed : 22/11/2001 16.24.33
Analysis Method : 
Last changed : 22/11/2001 16.24.33

External Standard Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=365.8 Ref=off

RetTime Type Area Amt/Area Amount Grp Name
[min] [mAU*s] [ng inj.]
7.652 0.0000 0.0000 OLAQ-olaquindox

Results obtained with enhanced integrator!

1 Warnings or Errors :
Warning : Calibrated compound(s) not found
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 22
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 - 2nd round
Lab-name: 
Contact person: 
e-mail: 
fax: 
telephone: 
Date of analysis: 01/11/15 01/11/20

<table>
<thead>
<tr>
<th>Analyte: OLAQUINDOX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample code</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td>225361</td>
</tr>
<tr>
<td>225373</td>
</tr>
<tr>
<td>225424</td>
</tr>
<tr>
<td>555416</td>
</tr>
</tbody>
</table>
CANFAS COLLABORATIVE STUDIES - 2nd round - NOVEMBER 2001

ADDITIVE: OLAQUINDOX

Annex 4 - Questionnaire

Laboratory: .......................................................... Contact person: ..........................................................

Date(s) of analysis: 01.11.15 01.11.20

Chromatographic conditions:

- Column:
  - □ As described in the method
  - □ Other: Hypersil C18 0.05 R.O.S. 5μm 250 x 4.6 mm

- Mobile phase:
  - □ As described in the method
  - □ Other: ..........................................................

- Flow-rate: .............. mL/min
- Injection volume: ........... μl
- Retention time of olaquindox: ........... min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample (from your own collection and to be used for recovery purposes)

Please indicate the olaquindox peak with an arrow

Recovery results:

- Percentage recovery: 101. %
- Single / duplicate determinations: □ single □ duplicate
  - If duplicate, please give both percentages: 105. % and 97. %
- Spiking level: 2.2 / 2.3 mg/kg
NEW TIMED EVENTS FROM BAYONOX

****** EXTERNAL STANDARD TABLE ******

***************** 11-17-2001 09:23:27 Version 5.1 ********************

* Sample Name: blank  
* Date: 11-11-2001 13:29:09  
* Interface: 0  
* Starting Peak Width: 2  

Data File: D:bayo07

Version: 5.1

Method: BAYONOX 11-17-2001 08:50:10 # 287

Operator ann Channel#: 0  
Vial#: 1

Cycle#: 1

Threshold: 1  
Area Threshold: 100

Starting Delay: 0.00

Ending retention time: 12.00

Area reject: 1000  
One sample per 0.200 sec.

Amount injected: 50.00

Sample Weight: 1.00000

Dilution factor: 1.00

PEAK RET PEAK CONCENTRATION in NORMALIZED AREA/ REF % DELTA

NAME TIME NAME ug/ml CONC AREA REF HEIGHT HEIGHT BL PEAK RET TIME CONC/

TOTAL AMOUNT = 0.0000

PEAKS NOT FOUND IN THIS RUN

NAME ADJUSTED RET.TIME. REFERENCE PEAK

ola 7.89

Data File = D:bayo07.PTS  
Printed on 11-17-2001 at 09:23:31

Start time: 0.00 min.  
Stop time: 15.00 min.  
Offset: 0 mv.

Low Value: 0 uv  
High Value: 222486 uv  
Scale factor: 8.0

Blank
### External Standard Table

**Sample Name:** prov nr 2  
**Date:** 11-16-2001 13:21:20  
**Method:** D:BAYONOX 11-17-2001 09:25:41 # 232  
**Interface:** 0  
**Cycle#:** 1  
**Operator ann Channel#:** 0  
**Vial#:**  
**Starting Peak Width:** 15  
**Threshold:** 1  
**Area Threshold:** 200  
**Starting Delay:** 0.00  
**Area reject:** 200  
**Amount injected:** 50.00  
**Sample Weight:** 1.00000

<table>
<thead>
<tr>
<th>TIME</th>
<th>NAME</th>
<th>CONCENTRATION in ug/ml</th>
<th>NORMALIZED</th>
<th>AREA/HEIGHT BL</th>
<th>REF PEAK</th>
<th>% DELTA RET TIME</th>
<th>CONC/AREA</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.151</td>
<td>cla</td>
<td>0.5100</td>
<td>100.0000%</td>
<td>33455</td>
<td>2147</td>
<td>15.6</td>
<td>1</td>
</tr>
</tbody>
</table>

**TOTAL AMOUNT =** 0.5100

---

**Notes:** times, and heights stored in: D:BAYO028.ATB  
**Data File = D:BAYO028.PTS**  
Printed on 11-17-2001 at 09:25:50  
**Start time:** 0.00 min.  
**Stop time:** 15.00 min.  
**Offset:** 0 mv.  
**Low Value:** 0 uv  
**High Value:** 99159 uv  
**Scale factor:** 8.0

---

**Sample:** 225373
**EXTERNAL STANDARD TABLE**

---

**Sample Name:** prov nr 4  
**Date:** 11-16-2001 13:48:11  
**Method:** D:BAYONOX 11-17-2001 09:25:41  
**Interface:** 0  
**Cycle #:** 1  
**Operator ann Channel #:** 0  
**Vial #:** 25  
**Starting Peak Width:** 15  
**Threshold:** 1  
**Area Threshold:** 200

<table>
<thead>
<tr>
<th>NAME</th>
<th>CONCENTRATION</th>
<th>NORMALIZED</th>
<th>AREA</th>
<th>HEIGHT</th>
<th>HEIGHT BL</th>
<th>PEAK</th>
<th>RET TIME</th>
<th>CONC/</th>
<th>% DELTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>olA</td>
<td>1.9754</td>
<td>100.0000%</td>
<td>129585</td>
<td>8302</td>
<td>15.61</td>
<td>1</td>
<td>0</td>
<td>1.524</td>
<td></td>
</tr>
</tbody>
</table>

---

**TOTAL AMOUNT = 1.9754**

---

*Sample times, and heights stored in: D:BAYO030.ATB  
Data File = D:BAYO030.PTS  
Printed on 11-17-2001 at 09:26:46  
Start time: 0.00 min.  
Stop time: 15.00 min.  
Offset: 0 mv.  
UV Value: 0 uv  
High Value: 89418 uv  
Scale factor: 8.0  
Sample 225424*
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 23
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 - 2nd round
Lab-name: __________________________
Contact person: _______________________
e-mail: _____________________________
fax: ________________________________
telephone: __________________________
Date of analysis: 15-12-2001
Analyte: OLAQUINDOX

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Unit</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>235325</td>
<td></td>
<td>2.35</td>
<td>2.46</td>
</tr>
<tr>
<td>235310</td>
<td></td>
<td>2.43</td>
<td>2.30</td>
</tr>
<tr>
<td>235342</td>
<td></td>
<td>8.55</td>
<td>8.60</td>
</tr>
<tr>
<td>235365</td>
<td></td>
<td>9.34</td>
<td>9.50</td>
</tr>
</tbody>
</table>
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 24
CANFAS COLLABORATIVE STUDIES - 2ND ROUND - NOVEMBER 2001

ANNEX 2 - Report form

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 - 2nd round
Lab-name: 
Contact person: 
e-mail: 
fax: 
telephone: 
Date of analysis: 12 dec 2001

Analyte: OLAQUINDOX

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>245307</td>
<td>8,89</td>
<td>7,99</td>
</tr>
<tr>
<td>245326</td>
<td>2,10</td>
<td>2,01</td>
</tr>
<tr>
<td>245410</td>
<td>2,09</td>
<td>2,01</td>
</tr>
<tr>
<td>245426</td>
<td>8,40</td>
<td>8,69</td>
</tr>
</tbody>
</table>
CANFAS COLLABORATIVE STUDIES - 2nd round - NOVEMBER 2001

ADDITIVE: OLAQUINDOX

Annex 4 - Questionnaire

Laboratory: ..........................................
Contact person: ..........................................

Date(s) of analysis: 18th December 2001

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ☐ Other: C18, 5 μm, 250 x 4.6 mm, Lichrospher X-Terra.

- Mobile phase:
  - ☐ As described in the method
  - ☐ Other: ..........................................

- Flow-rate: ... ml/min
- Injection volume: ... μl
- Retention time of olaquindox: ... min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample (from your own collection and to be used for recovery purposes)

Please indicate the olaquindox peak with an arrow

Recovery results:

- Percentage recovery: .... %
- Single / duplicate determinations: ☐ single ☑ duplicate
  If duplicate, please give both percentages: ... % and ... 
- Spiking level: ... mg/kg
**RING-TEST CANFAS OLAQUINDOX**

**Injection Date**: 12/12/01 16.56.48
**Sample Name**: Location: Vial 1
**Acq. Operator**: (modified after loading)

**External Standard Report**

---

**Sorted By**: Signal  
**Calib. Data Modified**: 12/12/01 13.11.35  
**Multiplier**: 1.0000  
**Dilution**: 1.0000

**Signal 1**: DAD1 A, Sig=380,4 Ref=550,100  
**RatTime** | **Type** | **Area** | **Amt/Area** | **Amount** | **Grp Name**  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>[min]</td>
<td>[mAU's]</td>
<td>[ng/ul]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| 9.870 PB | 177.15282 | 1.00387e-2 | 1.77838 | olaquindox

**Totals**: 1.77838

Results obtained with enhanced integrator!

*** End of Report ***
Injection Date: 12/12/01 12:59.29
Acq. Operator: 
Acq. Method: C:\HPCE\METHODS\CANTO.M
Last changed: 12/12/01 12:59.47 (modified after loading)
Analysis Method: C:\HPCE\METHODS
Last changed: 12/12/01 13:11.24 (modified after loading)

Sorted By: Signal
Calib. Data Modified: 12/12/01 12:59.44
Multiplier: 1.0000
Dilution: 1.0000

Signal 1: DAUI A, Sig=360.4 Ref=550.100

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>9.091</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>olaquindox</td>
</tr>
</tbody>
</table>

Totals:

0.00000

Results obtained with enhanced integrator!

Warnings or Errors:

Warning: Calibrated compound(s) not found

Instrument 1 12/12/01 13:11.25
APPENDIX 5

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)**

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>255349</td>
<td>2,88</td>
<td>2,86</td>
</tr>
<tr>
<td>255306</td>
<td>10,25</td>
<td>10,33</td>
</tr>
<tr>
<td>255383</td>
<td>2,88</td>
<td>3,02</td>
</tr>
<tr>
<td>255411</td>
<td>10,50</td>
<td>10,56</td>
</tr>
</tbody>
</table>
CANFAS COLLABORATIVE STUDIES - 2nd round - NOVEMBER 2001

ADDITIVE: OLAQUINODOX

Annex 4 - Questionnaire

Laboratory:
Contact person:
Date(s) of analysis:

Chromatographic conditions:

- Column:
  - [ ] As described in the method
  - [ ] Other: RP C18 LIChROCAP 750-4 (5μm) MERCK

- Mobile phase:
  - [ ] As described in the method
  - [ ] Other: H2O, 0.01M pD = 2.1 (DEA)/ACN 80% GRADIENT LINEAR

- Flow-rate: ... ml/min
- Injection volume: ... μl
- Retention time of olaquindox: ... min

Chromatograms: Please include representative chromatograms etc.

- Blind positive feed samples
- Blind blank feed sample (from your own collection and to be used for recovery purposes)
Please indicate the olaquindox peak with an arrow

Recovery results:

- Percentage recovery: ...%
- Single / duplicate determinations: [ ] single [ ] duplicate
  - If duplicate, please give both percentages: ...% and ...
- Spiking level: ... mg/kg
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 26
CANFAS COLLABORATIVE STUDIES - 2ND ROUND - NOVEMBER 2001

ANNEX 2 - Report form

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 - 2nd round
Lab-name: 
Contact person: 
Date of analysis: 19-11-2001
Analyte: OLAQUINDOX

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>265390</td>
<td>7,32</td>
<td>7,76</td>
</tr>
<tr>
<td>265398</td>
<td>7,77</td>
<td>7,85</td>
</tr>
<tr>
<td>265404</td>
<td>2,20</td>
<td>2,19</td>
</tr>
<tr>
<td>265413</td>
<td>2,25</td>
<td>2,25</td>
</tr>
</tbody>
</table>
ANNEX 4 - QUESTIONNAIRE

Laboratory: .................................................................
Contact person: ..............................................................

Date(s) of analysis: 19/11/2001

Chromatographic conditions:
• Column:
  □ As described in the method
  □ Other: SPHERisorb ODS 2 twin 250 x 4.6 mm
• Mobile phase:
  □ As described in the method
  □ Other: .................................................................
• Flow-rate: ........ ml/min
• Injection volume: ........ μl
• Retention time of olaquindox: ........ min

Chromatograms: Please include representative chromatograms of:
• Blind positive feed samples
• Blind blank feed sample (from your own collection and to be used for recovery purposes)
Please indicate the olaquindox peak with an arrow

Recovery results:
• Percentage recovery: ........ %
• Single / duplicate determinations: □ single □ duplicate
• If duplicate, please give both percentages: ........ % and ........ %
• Spiking level: ........ mg/kg
Sample Name: B3014229B

Data File: C:\TC4\CANFAS\OLAQUI~1\REPEAT~1\DATA024.RAW

Sequence File: C:\TC4\CANFAS\OLAQUI~1\REPEAT~1\RPTTRIAL.SEQ

Instrument: BOX_0

Sample Amount: 1.0000

Dilution Factor: 1.00

Peak time and area data for the sample:

<table>
<thead>
<tr>
<th>Peak</th>
<th>Time [min]</th>
<th>Area [µV·s]</th>
<th>Height [µV]</th>
<th>Area [%]</th>
<th>Norm. Area [%]</th>
<th>BL Area/Height [s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.018</td>
<td>203243.24</td>
<td>9421.37</td>
<td>100.00</td>
<td>100.00</td>
<td>*BB 21.57</td>
</tr>
<tr>
<td>0</td>
<td>9.862</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

Missing Component Report

Component: Olaquindox

Expected Retention (Calibration File): 9.862
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 29
CANFAS COLLABORATIVE STUDIES - 2ND ROUND - NOVEMBER 2001

ANNEX 2 - Report form

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 - 2nd round
Lab-name: 
Contact person: 
e-mail: 
fax: 
telephone: 

Date of analysis: 21_11_01

Analyte: OLAQUINDOX

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>295319</td>
<td>6,49</td>
<td>6,53</td>
</tr>
<tr>
<td>295354</td>
<td>1,66</td>
<td>1,61</td>
</tr>
<tr>
<td>295356</td>
<td>1,90</td>
<td>1,86</td>
</tr>
<tr>
<td>295418</td>
<td>5,70</td>
<td>5,97</td>
</tr>
</tbody>
</table>
CANFAS COLLABORATIVE STUDIES – 2nd round – NOVEMBER 2001

ADDITIVE: OLAQUINDOX

Annex 4 - Questionnaire

Laboratory: .................................................................
Contact person: ..........................................................

Date(s) of analysis: ..........01...11...22 ..................................................

Chromatographic conditions:
- Column:
  - ☐ As described in the method
  - ☑ Other: N.Ax. = .8 µm. £.18 µm. 4 μm. 4.6 x 25 cm.
- Mobile phase:
  - ☑ As described in the method
  - ☐ Other: .................................................................
- Flow-rate: ..........1.c.5. ml/min
- Injection volume: ..........1.c.µl
- Retention time of olaquindox: ......2.3. min

Chromatograms: Please include representative chromatograms of:
- Blind positive feed samples
- Blind blank feed sample (from your own collection and to be used for recovery purposes)

Please indicate the olaquindox peak with an arrow

Recovery results:
- Percentage recovery: ..........% 
- Single / duplicate determinations: ☐ single ☑ duplicate
- If duplicate, please give both percentages: 1.0. % and 1.0. %
- Spiking level: ..........µg/kg
Olaquindox Report

Sample Name: Spiked blank II 2.5 mg/kg
Sample Type: Unknown
Vial: 35
Injection #: 2
Injection Volume: 100.00 µl
Run Time: 15.0 Minutes
Sample Set Name: OLAQUINDOX Nov 2001

Acquired By: System
Date Acquired: 21-11-2001 22:04:34
Acq. Method Set: Olaquindox
Date Processed: 22-11-2001 10:49:10
Processing Method: 
Proc. Chnl. Descr.: PDA 380,0 nm

Printed 10:55:25 05-12-2001
Sample Name: olaquindox
Sample Type: Unknown
Vial: 26
Injection #: 1
Injection Volume: 100.00 μl
Run Time: 10.0 Minutes
Sample Set Name: olaquindox 22_01

Acquired By: System
Date Acquired: 22-11-2001 15:33:52
Acq. Method Set: Olaquindox
Date Processed: 23-11-2001 11:55:47
Proc. Chnl. Descr.: PDA 380,0 nm

<table>
<thead>
<tr>
<th>Name</th>
<th>RT</th>
<th>Area</th>
<th>Height</th>
<th>Amount</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>olaq</td>
<td>7.331</td>
<td>275360</td>
<td>13054</td>
<td>1.140</td>
<td>μg/ml</td>
</tr>
</tbody>
</table>

Printed 10:54:40 06-12-2001
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms

of partner 31
CANFAS COLLABORATIVE STUDIES - 2ND ROUND - NOVEMBER 2001

ANNEX 2 - Report form

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:  

Date of analysis: 27-11-2001

Analyte: OLAQUINDOX

<table>
<thead>
<tr>
<th>Unit Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>315359</td>
<td>8,71</td>
<td>9,09</td>
</tr>
<tr>
<td>315399</td>
<td>9,47</td>
<td>9,40</td>
</tr>
<tr>
<td>315414</td>
<td>2,70</td>
<td>3,10</td>
</tr>
<tr>
<td>315429</td>
<td>2,37</td>
<td>2,57</td>
</tr>
</tbody>
</table>
CANFAS COLLABORATIVE STUDIES - 2nd round - NOVEMBER 2001

ADDITIVE: OLAQUINDOX

Annex 4 - Questionnaire

Laboratory: .................................................................
Contact person: ..............................................................

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

Chromatographic conditions:

- Column:
  - ☑ As described in the method
  - ☐ Other: .................................................................

- Mobile phase:
  - ☑ As described in the method
  - ☐ Other: .................................................................

- Flow-rate: ....1.5.... ml/min
- Injection volume: .....1.00....μl
- Retention time of olaquindox: ....1.2....min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample (from your own collection and to be used for recovery purposes)

Please indicate the olaquindox peak with an arrow

Recovery results:

- Percentage recovery: ...85.5%...
- Single / duplicate determinations: ☑ single ☐ duplicate
  - If duplicate, please give both percentages: ...85.3% and 84.7%...

Spiking level: ..7.5... mg/kg
Sample Name: 6.1.2.0.1:D19
Instrument Name: HPLC-5
Sample Amount: 1,000000
Cycle: 6

Date: 13-12-01 9:35:33
Data Acquisition Time: 27-11-01 16:40:05
Operator: A
Dilution Factor: 1,000000

Result File
Sequence File

**olaquindox**

<table>
<thead>
<tr>
<th>Peak Component Name</th>
<th>Time [min]</th>
<th>Area [μV-s]</th>
<th>Height [μV]</th>
<th>Area [%]</th>
<th>Norm. Area [%]</th>
<th>BL Area/Height [s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>olaquindox</td>
<td>7,185</td>
<td>0,00</td>
<td>0,00</td>
<td>0,00</td>
<td>0,00</td>
<td>0,00</td>
</tr>
</tbody>
</table>

**Missing Component Report**

Component | Expected Retention (Calibration File)  
olaquindox | 7,185 |
Component Report

Component Expected Retention (Calibration File)

All components were found
### Software Version
6.1.2.0.1:D19

### Sample Name
004275-a

### Instrument Name
HPLC-5

### Rack/Vial
0/0

### Sample Amount
1.000000

### Cycle
16

### Date
13-12-01

### Data Acquisition Time
9:37:42

### Channel
A

### Operator

### Dilution Factor
1.000000

---

**Time [min]**

<table>
<thead>
<tr>
<th>Peak Component</th>
<th>Time [min]</th>
<th>Area [µV-s]</th>
<th>Height [µV]</th>
<th>Area [%]</th>
<th>Norm. Area [%]</th>
<th>BL Area</th>
<th>Area/Height [s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 olaquindox</td>
<td>7.259</td>
<td>36148,25</td>
<td>1917,65</td>
<td>100,00</td>
<td>100,00</td>
<td>BB</td>
<td>18,8503</td>
</tr>
</tbody>
</table>

**Missing Component Report:**

**Component Expected Retention (Calibration File):**

All components were found
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 32
### CANFAS

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

<table>
<thead>
<tr>
<th>Subtitle:</th>
<th>Task 4 COLLABORATIVE STUDY - 2nd round</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab-name:</td>
<td></td>
</tr>
<tr>
<td>Contact person:</td>
<td>e-mail:</td>
</tr>
<tr>
<td>Date of analysis:</td>
<td>12-11-2001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analyte:</th>
<th>OLAQUINDOX</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Unit (mg/kg)</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>325305</td>
<td>8,77</td>
<td>8,37</td>
<td></td>
</tr>
<tr>
<td>325364</td>
<td>3,16</td>
<td>2,81</td>
<td></td>
</tr>
<tr>
<td>325375</td>
<td>9,05</td>
<td>9,05</td>
<td></td>
</tr>
<tr>
<td>325409</td>
<td>3,14</td>
<td>2,69</td>
<td></td>
</tr>
</tbody>
</table>
Date(s) of analysis: 12/11/2001

Chromatographic conditions:

* Column:
  * ☐ As described in the method
  * ☑ Other: LiChrospher ® RP-select B (5μm), 250 x 4 mm
* Mobile phase:
  * ☐ As described in the method
  * ☑ Other: 
* Flow-rate: 1.8 ml/min
* Injection volume: 70 (μL)
* Retention time of olaquindox: 4.14 min

Chromatograms: Please include representative chromatograms of:

* Blind positive feed samples
* Blind blank feed sample (from your own collection and to be used for recovery purposes)

Please indicate the olaquindox peak with an arrow

Recovery results:

* Percentage recovery: 83.3 %
* Single/duplicate determinations: ☑ single  ☑ duplicate
* If duplicate, please give both percentages: 83.61% and 82.89%
* Spiking level: 2.5 mg/Kg
Injection Date : 11/12/01 2:29:51 PM  Seq. Line : 1
Sample Name : 325364  Vial : 1
Acq. Operator :  Inj : 1

Inj Volume : 70 µl
Acq. Method : C:\HPCHEM\1\METHODS\OLAQUIN.M
Analysis Method : C:\HPCHEM\1\METHODS\OLAQUIN.M

DAD1 A, Sig=380,100 Ref=550,50 (12/12/01 OLAQUIND.D)

External Standard Report

Sorted By : Signal
Calib. Data Modified : 11/12/01 4:41:01 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=380,100 Ref=550,50

<table>
<thead>
<tr>
<th>RetTime</th>
<th>Type</th>
<th>Area</th>
<th>Amt/Area</th>
<th>Amount</th>
<th>Grp</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.095</td>
<td>MM</td>
<td>30.92293</td>
<td>2.04513e-2</td>
<td>6.32414e-1</td>
<td>Olaquindox</td>
<td></td>
</tr>
</tbody>
</table>

Totals : 6.32414e-1

Results obtained with enhanced integrator!
Sample Name: 325375 Vial: 7

Injection Date: 11/12/01 3:27:35 PM
Seq. Line: 7
Sample Name: 325375 Vial: 7
Inj Volume: 70 μl

Acq. Method: C:\HPCHEM\1\METHODS\OLAQUIN.M
Analysis Method: C:\HPCHPM\1\METHODS\OLAQUIN.M

Multiplier 1.0000
Dilution 1.0000

Signal 1: DAD1 A, Sig=380,100 Ref=550,50 (12112001\OLAQUI07.D)

<table>
<thead>
<tr>
<th>RetTime</th>
<th>Type</th>
<th>Area</th>
<th>Amt/Area</th>
<th>Amount</th>
<th>Grp Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.107 MM</td>
<td>90.84964</td>
<td>1.99336e-2</td>
<td>1.81096</td>
<td>Olaquindox</td>
<td></td>
</tr>
</tbody>
</table>

Totals: 1.81096

Results obtained with enhanced integrator!

Page 1 of 1
Injection Date: 11/12/01 4:57:15 PM    Seq. Line: 18
Sample Name: Blank    Vial: 13
Acq. Operator:        Inj: 1
Inj Volume: 70 µl
Acq. Method: C:\HPCEM\1\METHODS\OLAQUIN.M
Last changed:          (modified after loading)
Analysis Method: C:\HPCEM\1\METHODS\OLAQUIN.M
Last changed: 1/"      (modified after loading)

External Standard Report

Sorted By: Signal
Calib. Data Modified: 11/12/01 4:41:01 PM
Multiplier: 1.0000
Dilution: 1.0000

Signal 1: DAD1 A, Sig=380,100 Ref=550,50 (12112001\OLAQUI18.D)

RefTime Type Area Amt/Area Amount Grp Name
[min] [mAU*s] [µg/ml]

| 4.139 | - | - | Olaquindox |

Totals: 0.00000

Results obtained with enhanced integrator!
Sample Name: Spiked 1
Injection Date: 11/12/01 5:04:52 PM
Seq. Line: 19
Sample Name: Spiked 1
Vial: 14
Inj Volume: 70 μl
Acq. Operator: Inj: 1
Acq. Method: C:\HPCHEM\1\METHODS\OLAQUIN.M
Last changed: 01 (modified after loading)
Analysis Method: C:\HPCHEM\1\METHODS\OLAQUIN.M
Last changed: 01 (modified after loading)

External Standard Report

Sorted By: Signal
Calib. Data Modified: 11/12/01 4:41:01 PM
Multiplier: 1.0000
Dilution: 1.0000

Signal 1: DAD1 A, Sig=380,100 Ref=550,50

<table>
<thead>
<tr>
<th>RetTime</th>
<th>Type</th>
<th>Area</th>
<th>Amf/Area</th>
<th>Amount</th>
<th>Grp</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>[min]</td>
<td>[mAU's]</td>
<td>[μg/ml]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.119 MM</td>
<td>20.02315</td>
<td>2.08786e-2</td>
<td>4.18055e-1</td>
<td>Olaquindox</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Totals: 4.18055e-1

Results obtained with enhanced integrator!
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 33
Subtitle: Task 4 COLLABORATIVE STUDY - 2nd round
Lab-name: 
Contact person: 
e-mail: 
fax: 
telephone: 
Date of analysis: 11-7-2001

Analyte: OLAQUINDOX

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>335304</td>
<td>2.70</td>
<td>2.60</td>
</tr>
<tr>
<td>335308</td>
<td>9.10</td>
<td>8.80</td>
</tr>
<tr>
<td>335347</td>
<td>2.90</td>
<td>2.70</td>
</tr>
<tr>
<td>335362</td>
<td>8.90</td>
<td>8.80</td>
</tr>
</tbody>
</table>
CANFAS COLLABORATIVE STUDIES - 2nd round - NOVEMBER 2001

ADDITIVE: OLAQUINDOX

Annex 4 - Que

Laboratory: .... Contact person

Date(s) of analysis: 7/11/01

Chromatographic conditions:
- Column:
  - As described in the method
  - Other:
- Mobile phase:
  - As described in the method
  - Other:
- Flow-rate: ml/min
- Injection volume: ul
- Retention time of olaquindox: min

Chromatograms: Please include representative chromatograms of:
- Blind positive feed samples
- Blind blank feed sample (from your own collection and to be used for recovery purposes)
  Please indicate the olaquindox peak with an arrow

Recovery results:
- Percentage recovery: %
- Single / duplicate determinations: single duplicate
- If duplicate, please give both percentages: % and %
- Spiking level: mg/kg
Peak Results

<table>
<thead>
<tr>
<th>SampleName</th>
<th>Name</th>
<th>RT</th>
<th>Area</th>
<th>Height</th>
<th>Amount</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>6361/01</td>
<td>OLA</td>
<td>2.892</td>
<td>88145</td>
<td>9141</td>
<td>2.877</td>
<td>mg/kg</td>
</tr>
</tbody>
</table>

Sample Set Name: OLA07
User Name: RVSA
Current Date: 7/11/01
Current Time: 02:05:47
Sample Set Name: OLA07
User Name: RVSA

Current Date: 7/11/01
Current Time: 02:05:45

Spectrum Index Plot
OLA - 2.898

Peak Results

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Name</th>
<th>RT</th>
<th>Area</th>
<th>Height</th>
<th>Amount</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 6360/01</td>
<td>OLA</td>
<td>2.898</td>
<td>284980</td>
<td>32412</td>
<td>9.048</td>
<td>mg/kg</td>
</tr>
</tbody>
</table>
Sample Set Name OLA07
User Name RVSA

Current Date 7/11/01
Current Time 02:05:38

Spectrum Index Plot

0,00
0,10 0,20 0,30 0,40 0,50 0,60 0,70 0,80 0,90 1,00

Peak Results

<table>
<thead>
<tr>
<th>SampleName</th>
<th>Name</th>
<th>RT</th>
<th>Area</th>
<th>Height</th>
<th>Amount</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BLK</td>
<td>OLA</td>
<td>2,673</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Peak Results

<table>
<thead>
<tr>
<th>SampleName</th>
<th>Name</th>
<th>RT</th>
<th>Area</th>
<th>Height</th>
<th>Amount</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BLK + 5</td>
<td>OLA</td>
<td>159505</td>
<td>19958</td>
<td>5.114</td>
<td>mg/kg</td>
</tr>
</tbody>
</table>
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 34
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 - 2nd round
Lab-name: 
Contact person: 
Date of analysis: 08-01-2002

<table>
<thead>
<tr>
<th>Analyte: OLAQUINDOX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample code</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>345321</td>
</tr>
<tr>
<td>345366</td>
</tr>
<tr>
<td>345379</td>
</tr>
<tr>
<td>345386</td>
</tr>
</tbody>
</table>
Channel 2
KromaSystem 2000 Version 1.83 RESULT REPORT: INTEGRATION

SYS2 - OLAQ37.SMP (modified):
No. 08: 345321 10g/50ml
Channel 2: DETECT 332
No Text

Program File ...... OLAQ001 ......
Worksheet .......... OLAQ ......
Peak Table ......... OLAQUIND ......
Parameter Table .. OLAQUIND ......
Report File .......... ......
Document File ......... ......

<table>
<thead>
<tr>
<th>No.</th>
<th>PNo</th>
<th>Ret.Time</th>
<th>Type</th>
<th>Name</th>
<th>Area</th>
<th>Amount</th>
<th>Rel.Ar %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>8.58 MOD</td>
<td>olaquindox</td>
<td>4.4462e+000</td>
<td>8.5929e+000</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

Sample Report
Page 1 of 1
Printed: 21-03-02 9:21
KromaSystem 2000

Channel 2
KromaSystem 2000 Version 1.83 RESULT REPORT: INTEGRATION

SYS2 - OLAQ37.SMP (modified):
No. 10: 345366 10g/50ml
Channel 2: DETECT 332
No Text

Acquired: 08.01.02 12:51:31
Processed: 18.03.02 11:51

Program File OLAQ001
Worksheet OLAQ
Peak Table OLAQUIND
Parameter Table OLAQUIND
Report File
Document File

<table>
<thead>
<tr>
<th>No.</th>
<th>PNo</th>
<th>Ret.Time</th>
<th>Type</th>
<th>Name</th>
<th>Area</th>
<th>Amount</th>
<th>Rel.Ar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>8.49</td>
<td>MLR</td>
<td>olaquindox</td>
<td>1.2242e+000</td>
<td>2.3659e+000</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Sample Report Page 1 of 1 Printed: 21-03-02 9:22
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 35
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 - 2nd round

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Unit</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>355317</td>
<td></td>
<td>2,00</td>
<td>2,00</td>
</tr>
<tr>
<td>355322</td>
<td></td>
<td>2,00</td>
<td>2,00</td>
</tr>
<tr>
<td>355357</td>
<td></td>
<td>7,90</td>
<td>7,50</td>
</tr>
<tr>
<td>355406</td>
<td></td>
<td>7,60</td>
<td>7,70</td>
</tr>
</tbody>
</table>
ADDITIVE: OLAQUINDOX

Annex 4 - Questionnaire

Laboratory: ........ .................................................................
Contact person: .................................................................

Date(s) of analysis 12/13 - 11 - 2001 .................................................................

Chromatographic conditions:
- Column:
  - □ As described in the method .................................................................
  - □ Other: .................................................................
- Mobile phase:
  - □ As described in the method .................................................................
  - □ Other: .................................................................
- Flow-rate: ...... 1,5...... ml/min .................................................................
- Injection volume: ...... 50......μl .................................................................
- Retention time of olaquindox: ...... 4...... min .................................................................

Chromatograms: Please include representative chromatograms of:
- Blind positive feed samples .................................................................
- Blind blank feed sample (from your own collection and to be used for recovery purposes)

Please indicate the olaquindox peak with an arrow .................................................................

Recovery results:
- Percentage recovery: ...... 75......%
- Single / duplicate determinations: □ single □ duplicate .................................................................
- If duplicate, please give both percentages: ...... 75......% and ...... 75......%
- Spiking level: ...... 5...... mg/kg .................................................................
### UV-Detector Results

<table>
<thead>
<tr>
<th>Pk #</th>
<th>Retention Time</th>
<th>Area</th>
<th>Height</th>
<th>ESTD concentration</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olaquindox</td>
<td>1</td>
<td>7.20</td>
<td>31052</td>
<td>1070</td>
<td>1.95262 mg/kg</td>
</tr>
</tbody>
</table>
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 37
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 - 2nd round

Lab-name: 

Contact person: 

Date of analysis: 11-12-2001

Analyte: OLAQUINDOX

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>375311</td>
<td>2.02</td>
<td>1.92</td>
</tr>
<tr>
<td>375343</td>
<td>7.54</td>
<td>8.09</td>
</tr>
<tr>
<td>375387</td>
<td>1.87</td>
<td>1.88</td>
</tr>
<tr>
<td>375405</td>
<td>8.34</td>
<td>7.91</td>
</tr>
</tbody>
</table>
ADDITIVE: OLAQUINDOX

Annex 4 - Questionnaire

Laboratory: .............
Contact person: ....

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

Chromatographic conditions:
- Column:
  - ☐ As described in the method
  - ☐ Other: LICHROPLATE RP 18 S 100X5 mm, Prep (50 ml, 40 °C)
- Mobile phase:
  - ☐ As described in the method
  - ☐ Other: .................................................................
- Flow-rate: ............. ml/min
- Injection volume: ..50...μl
- Retention time of olaquindox: 11.5 min

Chromatograms: Please include representative chromatograms of:
- Blind positive feed samples
- Blind blank feed sample (from your own collection and to be used for recovery purposes)
Please indicate the olaquindox peak with an arrow

Recovery results:
- Percentage recovery: 71.5%
- Single/duplicate determinations: ☐ single ☐ duplicate
- If duplicate, please give both percentages: 79.9% and 75.5%
- Spiking level: 2.5... mg/kg
Analysis Report

Name: 881b
Description: 881b
Type: Sample
Injection Volume: 50.0 μL

Injection: 1 of 1
Injected On: 12-12-01 06:01:38

Acquisition Log
Column Pressure (PSI): 2992
Noise (microAU): 6e+01
Run-Time Messages: None

Column Temperature (C): N/A
Pump Flow Stability: 0.8
Drift (microAU/min): -1e+02

Signal 1: UV2000 A 380 nm
Calculation Type: External Standard (Height)

mV or mAU

11.536 olaquindox

37.5405

80.4940

UV2000 A 380 nm

Minutes

-
**Analysis Report**

Name: 079b  
Description: 079b  
Type: Sample  
Injection Volume: 50.0 μL

**Acquisition Log**
- Column Pressure (PSI): 295B
- Noise (microAU): 9e+01
- Run-Time Message: None

**Signal 1: UV2000 A 350 nm**
- Calculation Type: External Standard (Height)
- Calculation Type: mV or mAU

![Graph showing UV2000 A 350 nm signal](image-url)

**Injection:** 1 of 1  
**Injection On:** 12-12-01 03:25:00

**Pump Flow Stability:** 1.1  
**Column Temperature (C): N/A**

Olaquindox in Feed
Mode: Reprocessed Data
Original Results: CATSP\SYSTEM1\Data\111201olaqps.RES
Reprocessed Results: CATSP\SYSTEM1\Data\111201olaqps.RMS

Analysis Report

Name: bik feed
Description: bik feed
Type: Sample
Injection Volume: 50.0 μL

Injection: 1 of 1
Injected On: 12-12-01 00:17:07

Vial: A08

Acquisition Log
Column Pressure (PSI): 2806
Noise (microAU): 3e+01
Run-Time Messages: None

Column Temperature (C): N/A

Pump Flow Stability: 1.7

Signal 1: UV2000 A 380 nm
Calculation Type: External Standard (Height)

mV or mAU

0.808  1.151  2.117

Page 17
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 38
Development and Validation of HPLC-methods for the official control of Coccidiostats and Antibiotics used as Feed Additives (SMT4-CT98-2216)

<table>
<thead>
<tr>
<th>Subtitle:</th>
<th>Task 4 COLLABORATIVE STUDY - 2nd round</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab-name:</td>
<td></td>
</tr>
<tr>
<td>Contact person:</td>
<td>e-mail:</td>
</tr>
<tr>
<td></td>
<td>fax:</td>
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<td>telephone:</td>
</tr>
<tr>
<td>Date of analysis:</td>
<td>10-12-2001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analyte:</th>
<th>OLAQUINDOX</th>
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<table>
<thead>
<tr>
<th>Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>385336</td>
<td>7,82</td>
<td>6,86</td>
</tr>
<tr>
<td>385355</td>
<td>2,30</td>
<td>2,36</td>
</tr>
<tr>
<td>385369</td>
<td>2,60</td>
<td>2,22</td>
</tr>
<tr>
<td>385374</td>
<td>7,50</td>
<td>7,83</td>
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</table>
CANFAS COLLABORATIVE STUDIES - OLAQUINDOX II

Annex 4 - Questionnaire

Laboratory:
Contact Person:

Date(s) of analysis: 12/10/01

Chromatographic conditions:
• Column:
  □ As described in the method
  X Other: Symmetry® C-18, 150 x 2.1 mm, 3.5 μm
• Mobile phase:
  □ As described in the method
  X Other: Isocratic MeOH/Water (5:95)
• Flow-rate: 0.3 ml/min
• Injection volume: 10 μl
• Retention time of Carbadox: 12.9 min

Chromatograms: Please include representative chromatograms of:
• Blind positive feed samples
• Blind blank samples
  Please indicate the olaquindox peak with an arrow

Recovery results:
• Percentage recovery: 52.5%
• Single / duplicate determinations: □ single  X duplicate
• If duplicate, please give both percentages: 52% and 53%
• Speaking level: 2 mg/kg
blank

spiked blank (3.83 mg/kg)

standard olaquindox 1.92 ppm

385369/1

385336/6
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 40
CANFAS COLLABORATIVE STUDIES - 2ND ROUND - NOVEMBER 2001

ANNEX 2 - Report form

---

**Title:** 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 - 2nd round**

**Lab-name:**

**Contact person:**

**Date of analysis:** 26.11.-6.12.01

**Analyte:** OLAQUINDOX

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Result 1 (mg/kg)</th>
<th>Result 2 (mg/kg)</th>
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</thead>
<tbody>
<tr>
<td>405315</td>
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<td>2.62</td>
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<tr>
<td>405381</td>
<td>8.84</td>
<td>8.87</td>
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<td>405385</td>
<td>8.93</td>
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<td>405391</td>
<td>2.87</td>
<td>2.84</td>
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ADDITIVE: OLAQUINDOX

Annex 4 - Questionnaire

Laboratory: ..............................................................
Contact person: ..........................................................

Date(s) of analysis: 26 November - 6 December 2001

Chromatographic conditions:

- Column:
  - ☐ As described in the method
  - ☐ Other: C18 spherical 5 mm 3.9 x 150 mm; WATERS
- Mobile phase:
  - ☐ As described in the method
  - ☐ Other: ..............................................................
- Flow rate: ................ ml/min
- Injection volume: .................. µl
- Retention time of olaquindox: .................. min

Chromatograms: Please include representative chromatograms of:

- Blind positive feed samples
- Blind blank feed sample (from your own collection and to be used for recovery purposes)

Please indicate the olaquindox peak with an arrow

Recovery results:

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

CANFAS sample 405381
CANFAS sample 405315
Blank piglet feed
Spiked blank for recovery test 1
APPENDIX 5

Table with results, questionnaire (page 1) and chromatograms of partner 41
## CANFAS COLLABORATIVE STUDIES - 2ND ROUND - NOVEMBER 2001

ANNEX 2 - Report form

### CANFAS

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

<table>
<thead>
<tr>
<th>Subtitle:</th>
<th>Task 4 COLLABORATIVE STUDY - 2nd round</th>
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<tbody>
<tr>
<td>Lab-name:</td>
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<tr>
<td>Contact person:</td>
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<td></td>
<td>fax:</td>
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<td></td>
<td>telephone:</td>
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| Date of analysis: | 19-11-2001 |

### Analyte: OLAQUINDOX

<table>
<thead>
<tr>
<th>Sample code</th>
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<tbody>
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</tr>
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<td>415330</td>
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<td>415396</td>
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<td>415430</td>
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CANFAS COLLABORATIVE STUDIES - 2nd round - NOVEMBER 2001

ADDITIVE: OLAQUINDOX

Annex 4 - Questionnaire

Date(s) of analysis: 19.11.2001

Chromatographic conditions:
- Column:
  - ☑ As described in the method
  - ☐ Other: .................................................................
- Mobile phase:
  - ☑ As described in the method
  - ☐ Other: .................................................................
- Flow-rate: 1.5 ml/min
- Injection volume: 10.0 µl
- Retention time of olaquindox: 10.5 min

Chromatograms: Please include representative chromatograms of:
- Blind positive feed samples
- Blind blank feed sample (from your own collection and to be used for recovery purposes)

Please indicate the olaquindox peak with an arrow Sample H117 used for recovery purposes

Recovery results:
- Percentage recovery: 100.0%
- Single / duplicate determinations: ☐ single ☑ duplicate
- If duplicate, please give both percentages: 101.2% and 98.8%
- Spiking level: 2.5 mg/kg
Sample Information

SampleName  4118  
Vial  9  
Injection  1  
Injection Volume  100,00 ul  
Channel  486  
Run Time  15,0 Minutes  

Auto-Scaled Chromatogram

Peak Results

<table>
<thead>
<tr>
<th></th>
<th>Name</th>
<th>RT</th>
<th>Area</th>
<th>Height</th>
<th>Amount</th>
<th>Units</th>
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<tbody>
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# Sample Information

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Vial</th>
<th>Injection</th>
<th>Injection Volume</th>
<th>Channel</th>
<th>Run Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>4119</td>
<td>10</td>
<td>2</td>
<td>100,00 ul</td>
<td>486</td>
<td>15.0 Minutes</td>
</tr>
</tbody>
</table>

## Auto-Scaled Chromatogram

![Auto-Scaled Chromatogram](chart.png)

## Peak Results

<table>
<thead>
<tr>
<th>Name</th>
<th>RT</th>
<th>Area</th>
<th>Height</th>
<th>Amount</th>
<th>Units</th>
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<tbody>
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<td>281652</td>
<td>9010</td>
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