The implementation of a System Control the Safety Parameters of Ukrainian Honey

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Honey is one of few Ukrainian products of animal origin, which export to EU is allowed.

Manufacture and export of honey is an important branch of Ukrainian Agribusiness
Exports of Ukrainian honey steadily increased since 2003*

* dates from laboratory of SCIVP
In 2012, Ukraine exported to the U.S., EU and Russian Federation 15 000 tons of honey.
SOME HISTORY

In the course of 2003 the European Laboratory repeatedly detected in batches of export of Ukrainian honey chloramphenicol residues.

(As it turned out it was the batches of honey reexported from China)
Veterinary Service of Ukraine was forced to develop a system of measures for prevention of ingress of veterinary drug residues in exports honey.
On the implementation of state monitoring plan for determine the residual amounts of toxicants in honey for 2004
14.04.2004  No 7
To enforce the Plan
State monitoring of residues
of toxicants in honey for 2004

4. Control of exported batches of honey carry out only Central State Veterinary Laboratory or the State Scientific-Research Control Institute of Veterinary Medicinal Product and Feed Additives (Lviv).

5. Permitted only homogenized honey for export
SOME HISTORY

From 2004 to the present time was obligatory analysed all export batch of Ukrainian honey.
LEGISLATION

- Decision about registration (re-registration) of veterinary medicinal product, substances, feeds and feed additives in Ukraine (Order SCVM No 40 on 28.05.03)

- Council Regulation (EEC) No.2377/90 26.06.90
LEGISLATION

- Order SCBM Ukraine from 23.12.04 No 143 "On approval of the maximum residue limits of MRLs."

- Council Regulation (EEC) No.2377/90 26.06.90
LEGISLATION

• ORDER SCVM №15 04.03.02 "The prohibition of the use of some veterinary drugs."
  - nitrofurans;
  - ronidazole;
  - dapsone;
  - chloramphenicol;
  - dimetridazole;
  - chlorpromazine;
  - chloroform;
  - metronidazole.

• Annex IV Council Regulation (EEC) No.2377/90 26.06.90
  - chloramphenicol,
  - chloroform,
  - chlorpromazine,
  - colchicine,
  - dapsone,
  - dimetridazole,
  - metronidazole,
  - ronidazole
  - nitrofurans (including furazolidone), nifursol nitrofurantoin, and nitrofurazone
COMMISSION DECISION
of 29 April 2004
on the approval of residue monitoring plans submitted by third countries in accordance with Council Directive 96/23/EC

Once adopted measures Ukraine has the right to export honey to the EU
Each batch of Ukrainian honey obligatory must be controled of the state laboratory for absence of antibiotic residues

Laboratory of SCIVP controled about 70% of export batch of honey
Antibiotics containing preparations are not registered in EU for honeybees treatment, therefore LOD of necessary control methods must be in the range of 0.1-10 ppb.

The first step was to provide state and manufacturing laboratories with methods, which meet aforementioned requirements.
During 2002-2012 16 methods of drug residues control in honey (particularly, main groups of potentially dangerous antibiotics) were developed in SCIVP
New methods have been approved and introduced into the practice of Ukrainian state and manufacturing laboratories.

10 staffs of the state vet laboratory and State Standard laboratory
26 staffs of the commercial laboratory
• Since incoming inspection of primary products is necessary for honey exporters, fast methods (CHARMII, ELISA) for antibiotics control in honey were introduced in these laboratories.

• Use test kits for screening analysis of antibiotics residues in honey assisted exports of Ukrainian honey on the world market
Requirements for sensitivity methods of the state laboratories*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Limit</th>
</tr>
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<tbody>
<tr>
<td>chloramphenicol</td>
<td>&lt;0.3 ppb</td>
</tr>
<tr>
<td>streptomycin</td>
<td>&lt;10 ppb</td>
</tr>
<tr>
<td>tetracyclin</td>
<td>&lt;10 ppb</td>
</tr>
<tr>
<td>nitrofuran (AOZ)</td>
<td>&lt;0.6 ppb</td>
</tr>
<tr>
<td>sulfonamide (STZ)</td>
<td>&lt;3 ppb</td>
</tr>
</tbody>
</table>

*- Ukrainian national plan for control veterinary drug residues
SOME HISTORY

The ratio between positive and negative results
determination of chloramphenicol in exported honey
Dynamics decreasing the number of analysis for determination of chloramphenicol residues in honey
2. In order to promote exports of Ukrainian honey recommend SDVM Ukraine in beekeeping ban the use of therapeutic and prophylactic medicinal products containing antibiotics and sulfonamides.
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RESULTS

The efficiency of introduced control methods and experts training is confirmed by the fact that during 2008-2012 among 1239 batch of exported honey, received for the obligatory state control, none of them was detected to contain antibiotics.
In 20% of Ukrainian honey detected antibiotic residues*

* - dates from laboratory of company-exporters
Problems in the control of honey
- Minimum required performance limit (MRPL) applies to several substances prohibited or not authorised in food-producing animals in the EU e.g. chloramphenicol, nitrofurans (Decision 2002/657).

- MRPLs are ‘the minimum content of an analyte in a sample, which at least has to be detected and confirmed’. They are the reference point for action (‘Action levels’) when evaluating food consignments (Decision 2005/34/EC).

- MRPL: for chloramphenicol – 0,3 ppb; for nitrofurans – 1,0 ppb. (Decision 2003/181)
Routine methods analysis of antibiotics residues in honey

<table>
<thead>
<tr>
<th>Residues</th>
<th>Methods</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>chloramphenicol</td>
<td>LC/MS/MS, ELISA</td>
<td>0.1-0.2 ppb</td>
</tr>
<tr>
<td>streptomycin</td>
<td>LC/MS/MS, ELISA, CHARMII</td>
<td>1-5 ppb</td>
</tr>
<tr>
<td>tetracycllin</td>
<td>LC/MS/MS, ELISA, CHARMII</td>
<td>2-5 ppb</td>
</tr>
<tr>
<td>nitrofuranes</td>
<td>LC/MS/MS, ELISA, CHARMII</td>
<td>0.2-0.6 ppb</td>
</tr>
</tbody>
</table>
- Antimicrobial drugs are not authorised for the treatment of honey bees in the EU, as there are no EU MRL established for honey (Directive 2001/110)
- The RPA (Reference Points for Action) concept is described in Decision 2005/34 and to date RPAs are established in honey for substances e.g. chloramphenicol and nitrofurans.
- In the absence of MRLs or RPAs for many residues of pharmacologically active substances in honey, finding any confirmed residue concentration in honey results in the rejection of the consignment. (Regulation 470/2009)
Requirements for sensitivity methods of the trade partners in EU and USA

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>chloramphenicol</td>
<td>&lt;0,1 ppb</td>
</tr>
<tr>
<td>streptomycin</td>
<td>&lt;2,0 ppb</td>
</tr>
<tr>
<td>tetracyclin</td>
<td>&lt;2,0 ppb</td>
</tr>
<tr>
<td>nitrofuran (AOZ)</td>
<td>&lt;0,2 ppb</td>
</tr>
<tr>
<td>sulfonamide (STZ)</td>
<td>&lt;2,0 ppb</td>
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Sensitivity ELISA methods for analysis honey

<table>
<thead>
<tr>
<th>antibiotic</th>
<th>sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>chloramphenicol</td>
<td>25 ppt</td>
</tr>
<tr>
<td>streptomycin</td>
<td>5.0 ppb</td>
</tr>
<tr>
<td>tetracycline</td>
<td>6.0 ppb</td>
</tr>
<tr>
<td>nitrofuran (AOZ)</td>
<td>0.1 ppb</td>
</tr>
<tr>
<td>sulfonamide (STZ)</td>
<td>STZ-?</td>
</tr>
</tbody>
</table>
Acid hydrolysis of sulfonamides (SA)

The largest problem for the company's laboratories exporters is analysis of sulfonamides

CA + conjugate carbohydrates $\rightarrow$ carbohydrates $\rightarrow$ free SA
We know about 2500 compounds belonging to sulfonamides including about 25 are widely used in medicine and veterinary medicine.

![Chemical Structures of Sulfonamides](image-url)
In Ukraine in 99% of cases of detection sulfonamides residues in honey found sulfathiazole (STZ)*

*- dates from laboratory of SCIVP
ELISA Sulfonamide

Honey:
sensitivity – 2ppb;
recovery – <89%;
Specificity (STZ) – 32%;
ELISA Sulfonamide
(new method for honey sample
real honey confirmed HPLC/FLD)

Honey:
sensitivity – 2ppb;
recovery – 52%;
specificity – 30%;
ELISA Sulfonamide
(new method for honey sample
compare result real honey confirmed HPLC/FLD)

<table>
<thead>
<tr>
<th>No</th>
<th>STZ ppb</th>
<th>r-biopharm methods</th>
<th>New methods</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Blanc</td>
<td>0,562*</td>
<td>0,420</td>
</tr>
<tr>
<td></td>
<td>CV</td>
<td>1,1</td>
<td>2,8</td>
</tr>
<tr>
<td>2</td>
<td>2,4</td>
<td>0,506</td>
<td>1,28</td>
</tr>
<tr>
<td></td>
<td>CV</td>
<td>2,1</td>
<td>6,9</td>
</tr>
<tr>
<td>3</td>
<td>2,1</td>
<td>0,734</td>
<td>1,21</td>
</tr>
<tr>
<td></td>
<td>CV</td>
<td>1,6</td>
<td>3,9</td>
</tr>
</tbody>
</table>
Introduce new methods

Inter-laboratory testing system for laboratories of honey exporters, introduced by SCIVP in 2013, will considerably improve their effectiveness.
Exporters of honey have to control every batch of honey. For one batch (20 ton) they must perform at least 10 tests for all the above parameters. The cost of such tests is very high so the cost of analysis is a very important indicator.
Conclusions

The presented system allows you to effectively take control of honey but the financial responsibility entrust only on company exporters of honey. This limits the ability to export Ukrainian honey. A possible way out of the situation can become a registration of honey producers.