

Determination of Chloramphenicol and Nitrofuran Metabolites in Honey and Beeswax

Hans Wunsch, Markus Walter, Eckard Jantzen, Juergen Kuballa

GALAB Laboratories, Max – Planck – Str. 1, D – 21502 Geesthacht, Germany, Tel +49 (0) 4152 889400, Fax +49 (0) 4152 889401, www.galab.de, info@galab.de

Introduction

Antibiotics are mainly used in apicultures for the treatment of bacterial brood diseases like the American foulbrood. Chloramphenicol and nitrofurans are vet drugs used in the apicultures against these diseases. In Europe this practice is illegal. The compounds in question are included in Annex IV of the Council Directive 2377/90: no maximum residue limits (MRL) could be elaborated which means a zero tolerance in all foodstuffs of animal origin. The presence of these compounds at trace level and the complexity of the honey and beeswax matrix

requires very sensitive methods of determination. The poster shows the development and validation of sensitive methods for chloramphenicol and nitrofuran metabolites according to the Council Directive 96/23 EC and the DIN ISO 32645. Due to the zero tolerance level of these compounds only the LC-MS-MS technique offers the possibility to develop sensitive and specific residue methods to ensure the quality of apiculture products.

Experiments

Extraction/clean-Up of nitrofuran metabolites in honey:

- 1g honey
- internal standard solution
- derivatisation
- ethylacetate extraction
- LC-MS-MS

Three transitions for AOZ are shown in Fig.1

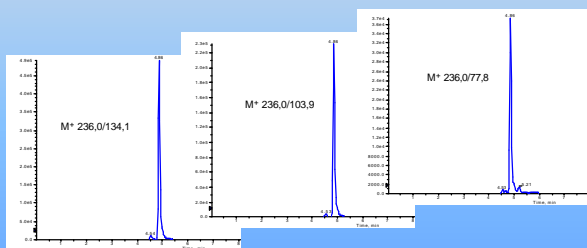


Fig. 1: real honey sample AOZ (conc. 1,0 µg/kg)

Extraction/clean-Up of chloramphenicol in honey/beeswax:

- 1g honey/beeswax
- internal standard solution
- addition of water/hexane
- acetonitrile extraction
- LC-MS-MS

Three transitions for chloramphenicol are shown in Fig.2

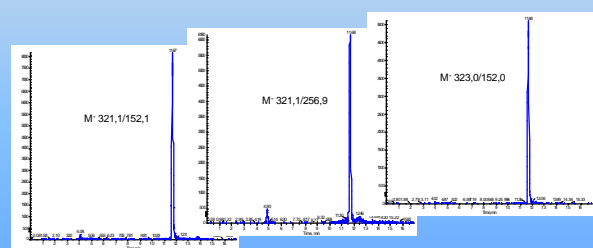


Fig. 2: real honey sample chloramphenicol (conc. 0,3 µg/kg)

Validation data

Method Validation According to DIN ISO 32645 and Council Directive 96/23 EC:

Method validation data for AOZ and chloramphenicol determination were calculated following DIN ISO 32645 and Council Directive 96/23 EC. Validation data are shown in Table 1.

The $cc\alpha$ and $cc\beta$ values were evaluated by equidistant sample calibration experiments. The application range for chloramphenicol in honey and beeswax is between 0,05 and 0,5 µg/kg and for AOZ between 0,1 and 1,0 µg/kg.

The recovery rates for nitrofuran metabolites and chloramphenicol are in the range of 67 to 92 %.

Table 1: Validation data of Nitrofuran Metabolites and Chloramphenicol in honey and beeswax						
compound	$cc\alpha$	$cc\beta$	LOD	IDL	precision	recovery
	[µg/kg]	[µg/kg]	[µg/kg]	[µg/kg]	VC [%]	[%]
honey						
Chloramphenicol	0,011	0,022	0,011	0,022	1,68	88,9
AOZ	0,049	0,098	0,049	0,098	2,06	76,7
AMOZ	0,074	0,146	0,074	0,146	1,54	75,4
AHD	0,208	0,416	0,208	0,416	1,03	66,8
SEM	0,176	0,352	0,176	0,352	3,69	72,5
beeswax						
Chloramphenicol	0,007	0,013	0,007	0,013	1,09	92,3

LOD = limit of detection
IDL = identification limit
LOD, IDL according to DIN ISO 32645
VC [%] = variation coefficient
 $cc\alpha$, $cc\beta$ according to Council Directive 96/23/EC

Proficiency testing

To confirm the developed methods we participate in proficiency testing for each compound. For the nitrofuran metabolite AOZ in honey the result is shown in table 2. (GDCh Gesellschaft Deutscher Chemiker July 2003)

Table 2:	Proficiency test Nitrofuran Metabolites				
matrix	honey				
compound	statistical values of 17 participants				
	min	max	mean	s	GALAB
AOZ [µg/kg]	0,67	2,37	1,45	0,38	1,49

The proficiency testing result for Chloramphenicol in honey is shown in table 3. (FAPAS March 2003)

Table 3:	Proficiency test Chloramphenicol				
matrix	honey				
compound	statistical values of 23 participants				
	min	max	mean	s	GALAB
CAP [µg/kg]	8,1	19,8	14,7	3,04	14,1

Conclusion

A highly sensitive LC-MS-MS method for the quantitative determination of nitrofuran metabolites and chloramphenicol in honey and beeswax has been developed and validated according the Council Directive 96/23 EC and DIN ISO 32645.

Successful participation in proficiency testing by Gesellschaft Deutscher Chemiker for nitrofuran metabolites and FAPAS for chloramphenicol.

GALAB Laboratories offers very sensitive methods for the determination of chloramphenicol and nitrofuran metabolites in honey and beeswax for a high product safety.