R-Biopharm AG

Simultaneous 5-in-1 sample preparation and multiplexed automated analysis by ChemWell® bioanalyzer of chloramphenicol and nitrofuran metabolites AOZ, AMOZ, AHD and SEM in shrimp

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Introduction

Chloramphenicol (CAP) and the nitrofuran antibiotics furazolidone, furaltadone, nitrofurantoin and nitrofurazone are worldwide banned for the application to animals used for food production, due to their potential carcinogenic and mutagenic characteristics. Nevertheless, they are still frequently and illegally used e.g. in aquaculture of shrimp. Residues bear a health risk for consumers and non-compliance with the established zero-tolerance levels may lead to severe penalties in export business. R-Biopharm developed a 5-in-1 sample preparation method to extract CAP and the 4 tissue-bound nitrofuran metabolites AOZ, AMOZ, AHD and SEM from shrimp simultaneously in one procedure. ELISA analysis can be either performed by manual pipetting or automated by ChemWell®, where CAP and all 4 nitrofuran metabolites can be analyzed in parallel.

Methods

For simultaneous sample preparation a special protocol was used. Concentration of CAP, AOZ, AMOZ, AHD and SEM was determined with enzyme immunoassay from R-Biopharm: RIDASCREEN® Chloramphenicol (Art. No. R1505), RIDASCREEN® Nitrofuran AOZ (Art No. R3703), AMOZ (Art No. R3711), AHD (Art. No. R3713) and SEM (Art. No. R3715). ChemWell® 2910 from Awareness Technology Inc. is a one-plate ELISA processor including fully automated pipetting with an integrated software to calculate results.

1 Aquaculture

Parent Drug
- Chloramphenicol
- Furazolidone
- Furaltadone
- Nitrofurantoin
- Nitrofurazone

Metabolites in shrimp
- CAP
- AOZ
- AMOZ
- AHD
- SEM

2 Sample preparation

1.5 h
3 h
- Homogenization
- Hydrolysis of tissue-bound metabolites with hydrochloric acid and derivatization of metabolites into nitrophenyl (NP-)derivates with 2-Nitrobenzaldehyde (2NBA)

1.5 h
- Extraction
- Evaporation
- Reconstitution

Sample Extract containing CAP, NP-AOZ, NP-AMOZ, NP-AHD, NP-SEM

3 Analysis

- Add Standard/Sample
- Add Enzyme Conjugate
- Add Antibody

1 h Incubation
- Washing
- Add Substrate-Chromogen

15 min Incubation
- Add Stop Solution
- Measurement

4 Data Evaluation

Evaluation of results with RIDA®SOFT WIN (Art. No. Z9996)

Results

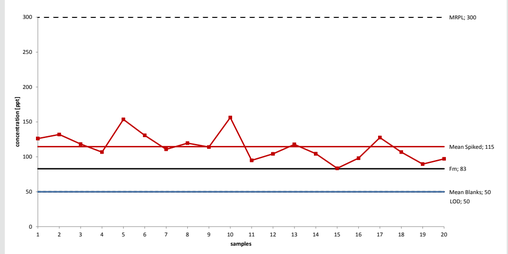
The limit of detection (LOD) of the enzyme immunoassays for CAP and all 4 nitrofuran metabolites is below the minimum required performance limit (MRPL) of 300 ng/kg for CAP and 1000 ng/kg for the nitrofurans. Recovery ranges between 69 and 123 %.

At a screening target concentration of 300 ng/kg for CAP, 200 ng/kg for AOZ and 1000 ng/kg for AMOZ, AHD and SEM, a clear discrimination between blank and spiked samples with no overlap can be observed. Consequently, detection capability (ccβ) is below the MRPL.

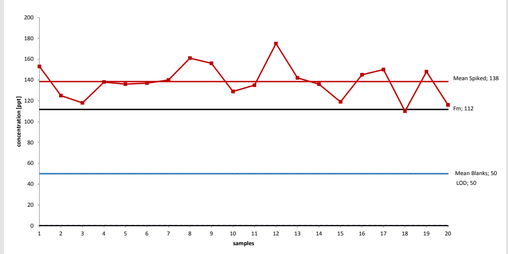
	RIDASCREEN® Chloramphenicol			RIDASCREEN® Nitrofuran AOZ			RIDASCREEN® Nitrofuran AMOZ			RIDASCREEN® Nitrofuran AHD			RIDASCREEN® Nitrofuran SEM		
	blank	spiked with Chloramphenicol at 150 ng/kg		blank	spiked with AOZ at 200 ng/kg		blank	spiked with AMOZ at 1000 ng/kg		blank	spiked with AHD at 1000 ng/kg		blank	spiked with SEM at 1000 ng/kg	
Sample	conc. [ng/kg]	conc. [ng/kg]	recovery [%]	conc. [ng/kg]	conc. [ng/kg]	recovery [%]	conc. [ng/kg]	conc. [ng/kg]	recovery [%]	conc. [ng/kg]	conc. [ng/kg]	recovery [%]	conc. [ng/kg]	conc. [ng/kg]	recovery [%]
1	< Std 2	126	84	< Std 2	153	77	131	973	97	< Std 2	1056	106	343	1418	142
2	< Std 2	132	88	< Std 2	125	63	84	634	63	< Std 2	756	76	174	1158	116
3	< Std 2	118	79	< Std 2	118	59	109	779	111	< Std 2	791	81	148	1254	142
4	< Std 2	107	71	< Std 2	138	69	75	657	66	< Std 2	763	76	69	1482	148
5	< Std 2	154	102	< Std 2	136	68	< Std 2	710	71	< Std 2	861	86	60	1251	125
6	< Std 2	131	87	< Std 2	137	69	< Std 2	877	88	< Std 2	976	98	251	1516	152
7	< Std 2	111	74	< Std 2	140	70	88	788	79	< Std 2	1256	126	127	1042	104
8	< Std 2	120	80	< Std 2	161	81	95	708	71	< Std 2	914	91	231	949	95
9	< Std 2	114	76	< Std 2	156	78	94	941	94	< Std 2	1004	100	338	1187	119
10	< Std 2	156	104	< Std 2	129	65	60	613	61	< Std 2	767	77	130	1195	120
11	< Std 2	95	63	< Std 2	135	68	< Std 2	786	79	< Std 2	761	76	101	1124	112
12	< Std 2	104	69	< Std 2	175	88	63	737	74	< Std 2	879	88	59	1277	128
13	< Std 2	118	79	< Std 2	142	71	< Std 2	779	78	< Std 2	856	86	106	1231	123
14	< Std 2	104	70	< Std 2	136	68	< Std 2	688	69	< Std 2	910	91	189	1047	105
15	< Std 2	84	56	< Std 2	119	60	< Std 2	627	63	< Std 2	796	80	117	903	90
16	< Std 2	98	65	< Std 2	145	73	62	949	95	< Std 2	1090	109	310	1428	143
17	< Std 2	128	85	< Std 2	150	75	75	1000	100	< Std 2	615	62	52	1137	114
18	< Std 2	107	71	< Std 2	110	55	91	670	67	< Std 2	794	79	76	1516	152
19	< Std 2	90	60	< Std 2	148	74	96	1010	101	< Std 2	994	99	148	1047	105
20	< Std 2	97	65	< Std 2	116	58	93	850	85	< Std 2	910	91	110	1254	125
Mean	< Std 2	115	76	< Std 2	138	69	87	789	81	< Std2	887	89	157	1221	123
SD*		19			16		19	131			146		92	181	
CV**		17%			12%			17%			16%			15%	
LOD***	50			50			145			200			434		

* SD = Standard Deviation
** CV = Coefficient of Variation
*** LOD = Limit of Detection
Std2 = Standard 2

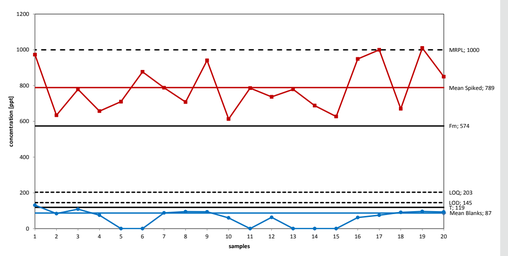
CAP



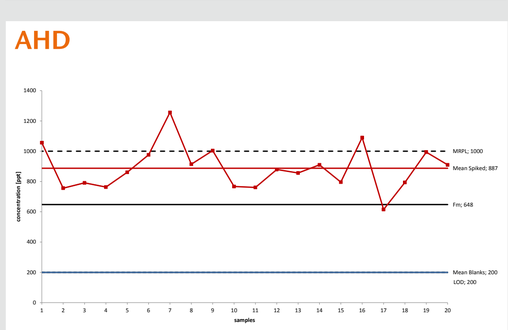
AOZ



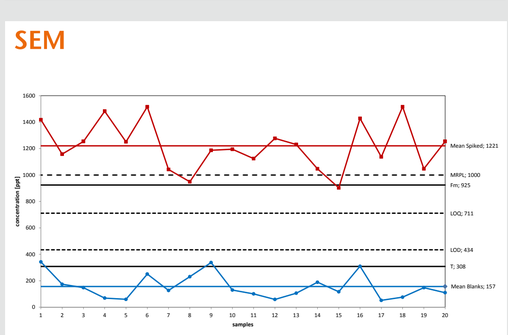
AMOZ



AHD



SEM



Blank Samples
Spiked Samples
MRPL = Minimum Required Performance Limit
Mean Blanks: Mean of Blank Samples
Mean Spiked = Mean of Spiked Samples
LOQ = Limit of Quantification = Mean Blank Samples + 9 x Standard Deviation of Blank Samples
LOD = Limit of Detection = Mean Blank Samples + 3 x Standard Deviation of Blank Samples
Fm = Cut-Off Factor = Mean Spiked Samples - 1,64 x Standard Deviation of Spiked Samples
T = Technical Threshold = Mean Blank Samples + 1,64 x Standard Deviation of Blank Samples

Conclusion

The simultaneous 5-in-1 sample preparation method in combination with automated multiplex analysis by ChemWell® 2910 offers a sensitive and legislation compliant method to screen shrimp for residues of the prohibited nitrofuran antibiotics and of chloramphenicol. In routine analysis, it offers a time and labor saving alternative to separate single analysis. For producers, exporters and processors of seafood, it can help to prevent penalties from violation of zero-tolerance levels. Overall, the new method improves general food safety and consumer protection.

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