

The Determination of Oxytetracycline Hydrochloride in Animal and Fish Feeds, Premixes and Animal Remedies by Liquid Chromatography with Fluorescence Detection: A Collaborative Study Report

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ABSTRACT:

A method for determining oxytetracycline in animal feed, fish feed and animal remedies at medicating and trace levels was studied. Oxytetracycline hydrochloride (OTC.HCl) is extracted from ground feed material in acid-methanol solution using mechanical agitation. After centrifugation for 5 minutes at 1230 g, an aliquot of the extract is diluted with water and/or acid-methanol so that the concentration of OTC.HCl is approximately the same as in the working standard, and the solutions contain at least 50 % water. Injectable animal remedy materials are diluted with water and/or extractant to reach the target concentration. The extracts are filtered and analyzed by reversed phase LC with fluorescence detection with excitation at 390 nm and emission at 512 nm. Twenty-eight test samples of medicated feeds, supplements and drug premixes, including four test samples for trace level analysis, were sent to 17 collaborators in Canada, The Netherlands, and the United States. Results were received from 11 laboratories. The RSDr values (within-laboratory repeatability) ranged from 1.26 %– 9.21 %, RSDR values (among-laboratory reproducibility) ranged from 2.14 % -12.9 % and HorRat values ranged from 0.54 to 3.02. The method is recommended for Official First Action.

INTRODUCTION:

Oxytetracycline hydrochloride (C₂₂H₂₄N₂O₉) is used as an aid in stimulating appetite, maintaining weight gains, and feed efficiency, in pigs, swine, beef cattle, chickens and turkeys. In addition it also serves as an aid in egg production, hatchability and eggshell quality during periods of stress and/or bacterial infections or potential infections in broiler, replacement and laying chickens, and turkeys. Oxytetracycline is also used as an aid in the prevention, treatment and reduction of losses due to bacterial infections in lambs, pigs and calves; as an aid to reduce the incidence of abortion in swine and bloat in young cattle; and for the treatment of various diseases in salmon and trout.

Oxytetracycline is a product of the metabolism of *Streptomyces rimosus* and is one of the family of tetracycline antibiotics. (See Figure 1.) The CAS name is: (4S, 4aR, 5S, 5aR, 6S, 12aS)-4-(dimethylamino)-1,4,4a, 5,5a, 6,11,12a-octahydro-3, 5,6,10,12,12a-hexahydroxy-6-methyl-1, 11-dioxo-2-naphthacene-carboxamide. Methodology is required to verify the levels of oxytetracycline in various feeds, supplements and premixes. Methodology is also required to determine contamination levels to verify clean out of manufacturing equipment for the prevention of cross contamination. Due to potential residues in tissues and animal products for use in food, withdrawal periods are required. The only current official method for oxytetracycline in animal feed is plate assay AOAC Official Method 968.50.

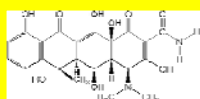


Figure 1. Structure of Oxytetracycline

The AOAC Agricultural Community, Feed Additives Group, identified the need for an Official HPLC method as a high priority method need.

The principle of the method under collaboration involves extracting oxytetracycline hydrochloride (OTC.HCl) from ground feed in acid-methanol solution using mechanical agitation. After centrifugation for 5 minutes at 2000 rpm, an aliquot of the extract is diluted with water and/or acid-methanol so that the concentration of OTC.HCl is approximately the same as in the working standard, and the solutions contain at least 50 % water. The extracts are filtered and analyzed by reversed phase HPLC with fluorescence detection. Injectable animal remedy solutions are diluted with water and/or extractant to reach the target concentration and are analyzed by the same method. The method is applicable to the analysis of animal feeds and mineral premixes containing levels ≥ 2 mg/kg, fish feed containing levels ≥ 10 mg/kg and animal remedy products.

SINGLE LABORATORY VALIDATION

Original method development data were published by Houglum et al "Assay of Oxytetracycline in Animal Feeds by Liquid Chromatography with Fluorescence Detection" published in the Journal of AOAC International Vol. 81, No. 5, 1998 (1). A subsequent Single Laboratory Validation was completed by Gita Nayeri of the Canadian Food Inspection Agency (2). Methods performance criteria as were developed by the AOAC Agricultural Community, Feed Additives and Contaminants Community and announced on their website http://www.aoac.org/Ag_Materials/additives/main.htm (3). Desired Community method performance and methods validation data were met in the SLV data.

COLLABORATIVE STUDY

The study is designed as described in the JAOAC (4) and in the Official Methods of Analysis, 18th Edition (2005), Appendix D: "Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis." (5). Twenty-eight test samples of medicated feeds, supplements and drug premixes, including four test samples for trace level analysis were sent to 17 collaborators in Canada, The Netherlands, and the United States. Collaborators were solicited from research, commercial, industry and regulatory laboratories. Participants received no compensation. Participating laboratories were required to demonstrate proficiency with the method on familiarization samples before attempting to analyze study samples.

The materials included used in this study are representative of animal feeds, premixes, fish feed and animal remedies that would typically contain oxytetracycline. The study materials were ground using a shearing or blending type mill to pass through a 2 mm sieve. All dry materials were split using a Fritsch Rotary Sample Divider Model Laborette 27. No further grinding was necessary by the participating laboratory. Study materials were packaged in polyethylene bags in ~25 to 30 g quantities. The milk replacer and injectable remedy solution required no grinding. Homogeneity tests were performed on study materials after splitting and bagging by checking five randomly chosen bags for OTC.HCl determinations. Samples were accepted as sufficiently homogenous if the coefficient of variation (CV) for the five subsamples was less than 2 %.

RESULTS

The study data were examined for evidence of individual systematic error using Cochran and Grubbs tests progressively, by the procedures described in the *Collaborative Study Guidelines* (4). Calculations for repeatability, r, and reproducibility, R, as defined by the guidelines were carried out after removal of outliers. HorRat values, the ratio of the RSDR to the predicted RSDR, were calculated. All outlier removal and statistics were calculated using AOAC Interlaboratory Statistical Program 2007 for Blind Duplicates software Version 1.14 provided by Joanna Lynch, Ithaca, NY. HorRat values between 0.5 and 1.5 are the limits for performance acceptability (4). The calculated method performance data (repeatability, reproducibility, and HorRat values) are shown in Tables 2007.XX. The data reported by the participating laboratories, are shown in Tables 1 and 2.

DISCUSSION

The results of analyses on medicated materials from 11 laboratories are shown in Table 3. The results of analyses from the same 11 laboratories on low and trace level materials are shown in Table 4. The statistical analysis is presented in Tables 2007.XX. The RSDr values (within-laboratory repeatability) ranged from 1.26 - 10.1%, RSDR values (among-laboratory reproducibility) ranged from 3.53% -17.3% and HorRat values ranged from 0.54 to 3.02. Eleven of the 14 HorRat values were under 2, the exceptions were injectable liquid remedy, Terramycin 100, and Terramycin 200 which were 2.68, 3.02, and 2.47 respectively. These materials are very clean materials and not subject to chromatographic interference. A factor potentially contributing to HorRats over 2 is the very high dilution factors for the highly concentrated materials. The authors believe that the largest source of variability in analyzing the injectable liquid remedy was the high viscosity of the material. Analysts not accustomed to pipetting them may not have allowed the pipette to drain sufficiently. The method has been revised to include more specific handling instructions for viscous materials. The authors suggest that the method is demonstrated as suitable for the wide range of materials under study.

RECOMMENDATIONS:

The data show that the method is valid for determining the presence and quantity of oxytetracycline in finished animal feed and fish feed, supplements, premixes, animal remedies (liquid and solid). It is also valid for determining the presence of oxytetracycline at residue levels in finished animal and fish feed. The collaborative study demonstrated the performance of the method was demonstrated from 4 to 200,000 mg/kg. It is recommended that the method be approved as AOAC Official First Action.

Table 1 Medicated Sample Data, (mg/g)

Lab #	# of Repts	Oxytetracycline Hydrochloride, mg/g										Oxytetracycline Base, mg/mL					
		Powdered Remedy		NeoOxy 1010		Terramycin MR		Terramycin 50		Salmon Feed		Terramycin 100		Injectable Liquid Remedy			
		1	2	3	9	4	14	7	12	10	11	19	20	26	27	15	21
1	2	55.0	53.4	22.1	21.6	215	211	108	108	11.4	11.6	209	212	409	396	209 ^a	214 ^a
2	2	59.0	61.5	22.9	23.1	288 ^a	226 ^a	112 ^b	109 ^b	11.5	11.6	213	226	424	406	183	180
4	2	51.7	52.9	21.1	21.4	209	212	104	103	12.2	12.0	169	166	390	391	193	189
6	2	55.1	56.2	21.0	20.7	208	206	101	104	11.3	11.5	206	206	405	401	182	182
7	2	54.0	54.8	20.5	21.2	207	210	108	102	11.9	11.6	186	192	385	380	202	194
8	2	52.0	50.8	20.1	20.2	213	197	98.2 ^b	94.5 ^b	11.5 ^a	10.1 ^a	166 ^a	213 ^a	346	332	189	199
9	2	54.0	52.7	21.2	20.9	215	212	105	102	11.2	10.8	212	211	388	384	191	192
10	2	51.8	52.3	20.5	20.7	204	202	104	105	11.0	11.1	186	187	322 ^a	226 ^a	182	162
11	2	53.6	52.1	20.4	20.6	200	212	106	101	11.2	10.9	205	196	392	386	170	171
12	2	49.1	49.6	20.6	20.0	202	206	105	106	11.7	12.0	196	201	390	385	184	191
13	2	56.0	59.9	21.5	21.1	213	212	106	103	12.9	12.6	191	201	353	387	196 ^a	95.4 ^a

^a Data excluded by Cochran's test, p=2.5% (1-tail)

^b Data excluded by Double Grubbs test, p=2.5% (2-tail), 1.25% (1-tail).

Table 2 Low and Trace Level Sample Data, (mg/kg)

Lab #	# of Repts	Oxytetracycline, mg/kg											
		AAFCO-200425 Beef Starter		AAFCO Catfish Feed		AAFCO-200430 Milk Replacer		Swine Feed		AAFCO-200331 Show Broiler		AAFCO Swine Feed	
		5	23	6	13	8	22	16	25	17	18	24	28
1	2	312	312	1110	1130	226	226	5.37	5.38	14.5	16.4	51.2	48.5
2	2	297	290	1870 ^a	1300 ^a	258 ^a	259 ^a	119 ^a	5.20 ^a	16.8	17.6	44.7	47.4
4	2	291	291	1070	1090	218	212	4.70	4.10	14.2	16.5	44.8	43.3
6	2	273	301	1040	1060	206	227	5.39	5.92	13.3	16.6	45.0	46.5
7	2	286	291	1020	1060	203	208	4.19	5.45	13.2	14.4	44.2 ^a	72.5 ^a
8	2	321	296	1000	1150	242 ^a	46.0 ^a	7.40 ^a	8.10 ^a	14.7	14.3	51.4	51.9
9	2	282	274	1060	1090	213	205	3.97	4.58	11.4	14.3	43.9	44.0
10	2	272	271	1030	1010	210	210	4.40	4.80	13.1	11.8	42.7	42.5
11	2	300	301	1100	1080	219	213	3.59 ^a	2.71 ^a	25.0 ^b	22.7 ^b	50.1 ^a	41.4 ^a
12	2	296	301	1060	1080	217	221	4.26	4.48	12.7	11.3	45.7	46.1
13	2	289	304	1140	1040	229	206	5.32	4.76	13.5	14.0	80.7 ^a	155 ^a

^a Data excluded by Cochran's test, p=2.5% (1-tail)

^b Data excluded by Single Grubbs test, p=2.5% (2-tail), 1.25% (1-tail).

^c Data excluded by Double Grubbs test, p=2.5% (2-tail), 1.25% (1-tail).

Table 2007.XX Interlaboratory Results Oxytetracycline in Animal Feed, Fish Feed, and Animal Remedies

Material	Mean mg/kg*	# of Labs (outliers)	Sr	RSD _r %	SR	RSD _R %	HorRat
Powdered Remedy	54000	11	1230	2.28	3200	5.93	1.91
NeoOxy 1010	21100	11	265	1.26	833	3.95	1.11
Terramycin MR	208000	10(1)	4680	2.25	5070	2.44	0.96
Terramycin 50	1040000	9(2)	2170	2.08	2230	2.14	0.76
Salmon Feed	11600	10(1)	181	1.56	570	4.92	1.26
Inject Liq Remedy	188mg/mL	10(1)	5843	3.11	13000	6.89	2.68
Terramycin 100	198000	10(1)	4600	2.32	15300	7.70	3.02
Terramycin 200	386000	10(1)	8520	2.21	22000	5.71	2.47
AAFCO-200425 Beef Starter	293	11	9.04	3.08	13.6	4.65	0.68
AFFCO- Catfish Feed	1070	10(1)	42.5	3.96	42.5	3.96	0.71
AAFCO-200430 Milk Replacer	215	9(2)	7.98	3.71	8.31	3.87	0.54
Swine Feed	4.82	8(3)	0.44	9.20	0.59	12.3	0.97
AAFCO-200331 Show Broiler	14.2	10(1)	1.31	9.21	1.84	12.9	1.20
AFFCO Swine Feed	46.2	9(2)	2.30	4.98	3.30	7.16	0.80

*unless otherwise indicated.

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