HPLC METHOD FOR THE ANALYSIS OF DEXAMETHASONE IN FEED AND WATER FOR LIVESTOCK

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Introduction

Dexamethasone (9-α-fluoro-16-α-methylprednisolone) is a synthetic glucocorticoid that is authorised for therapeutic use in veterinary medicine but its use as a growth promoting agent is banned in the European Union. Low concentrations of glucocorticosteroids are known to increase weight gain, to improve feed conversion and to have a synergetic effect with other molecules like beta-agonists or anabolic steroids. Due to these effects, dexamethasone has been illegally used to obtain an economic benefit through increased muscle development. A number of methods for the detection, determination and confirmation of dexamethasone in different biological matrices, like urine, faeces, liver, milk or feed, have been previously reported (Delahaut *et al.*, 1997; Creaser *et al.*, 1998; Stolker *et al.*, 2000; Draisci *et al.*, 2001; Cherlet *et al.*, 2004). Recently, the performance of methods and the criteria for the interpretation of test results of official control laboratories within the European Union has been regulated in the Decision 2002/657/EC (EC, 2002). The presented method, based on inmunoaffinity chromatography followed by Reverse phase HPLC with diode array detection at 242nm, has been validated according to this Decision.

Materials and Methods

Sample preparation: 2g of feed were weighed in a test tube and the internal standard (flumethasone) was added. After fortification and equilibration, the sample was extracted with 10mL of TBME (*tert*-butyl-methyl-ether), shaken for 20 min and centrifuged for 10 min at 2700rpm. The supernatant was collected in a clean glass tube, and the extraction was repeated. Both supernatants were loaded into an amino propyl (NH_2) cartridge (500mg) and eluted with 4 mL of methanol-water (80:20, v/v). The elutant was evaporated to dryness at 45°C under a stream of nitrogen.

Chromatography: The evaporated sample was resuspended and loaded into an immunoaffinity column, containing specific antibodies for dexamethasone. In the case of water samples (5mL), the internal standard (flumethasone) was added and then loaded in the immunoaffinity column. Corticosteroids were eluted with 4mL of ethanol:water (70:30 v/v), pH 5.0, and collected into a test tube that was evaporated to dryness at 45°C under nitrogen stream. Then, the evaporated sample was resuspended in 200 µL of mobile phase consisting of acetonitrile:mili-Q water (30:70), 20µL were injected into an Agilent series 1100 HPLC equipped with a diode-array detector. The column was a Synergi Max RP, 150mm x 4.6mm, from Phenomenex. The mobile phase, at a flow rate of 1mL per min, was a solution consisting in acetonitrile:mili-Q water (30:70) and the eluent was monitored at 242nm.

Results and Discussion

Stability: Dexamethasone solutions (10µg mL^{-1}) were kept under frozen storage (-20° C) up to 6 months and showed good stability for the full period of time.

Specificity: The method discriminated very well between the analyte (dexamethasone) and closely related substances, as can be appreciated in the chromatogram (see Figure 1) as they eluted at different retention times.

Recovery: The recovery was determined by experiments using a total of 24 fortified blank water samples. The recoveries, standard deviations and coefficients of variation (CV) were determined (see Table 1).

Repeatability: For feed, aliquots of the same sample were fortified at levels of 190, 285 and 380 ng mL^{-1} At each level, the analysis was performed with 6 replicates and the mean concentrations, standard deviations and coefficients of variation were determined (see Table 1).

Decision limit (CCα): 22 blank water/feed samples were analysed. The decision limit was set as 3 times the signal to noise ratio. This gives a CCα = 26 ng mL^{-1} for water and 190 ng mL^{-1} for feed.

Detection capability (CCβ): The value of the decision limit plus 1.64 times the standard deviation of the within-laboratory reproducibility of the measured content equals the detection capability. The obtained CCß was 30 ng mL^{-1} for water and 217 ng mL^{-1} for feed.

Within-laboratory reproducibility: Analysis were performed by different operators, days and reactives. The mean concentrations, standard deviations and coefficients of variation were determined (see table 1).

Table 1: Recovery, repeatability and within-laboratory reproducibility.

		Recovery			Repeatability			Within-lab- reproducibility		
	Level (ppb)	%	SD	CV (%)	Mean Conc. (ppb)	SD	CV (%)	Mean Conc. (ppb)	SD	CV (%)
Water	26	105.10	1.73	6.33	27.90	2.05	7.35	26.96	1.69	6.27
	39	98.50	1.08	2.80	44.94	0.73	1.62	39.61	2.89	7.30
	52	94.50	1.09	2.22	59.25	2.86	4.83	54.65	4.24	7.76
Feed	190	108.91	15.31	7.40	205.06	14.45	7.05	196.15	17.35	8.85
	285	118.22	7.22	2.12	314.14	18.97	6.04	314.14	18.97	6.04
	380	108.68	16.81	4.11	430.91	34.66	8.04	430.91	34.66	8.04

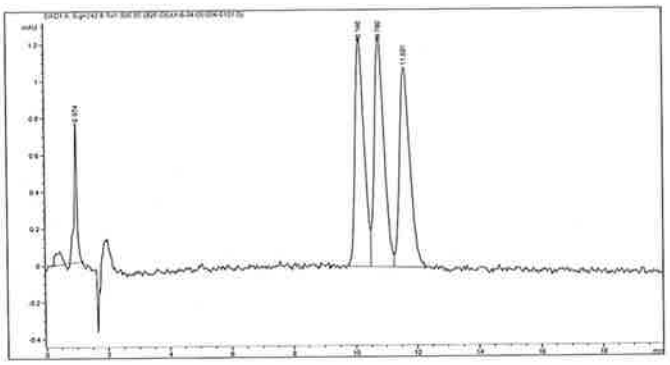

Figure 1: Chromatogram of dexamethasone and closely related substances for the specificity study. Retention times of 10.14, 10.78 and 11.60 min were for betamethasone, dexamethasone and flumethasone, respectively.

Conclusions

The method based on immunoaffinity chromatography followed by RP-HPLC for the analysis of dexamethasone in livestock drinking water and feed has been validated using water/feed fortified at levels up to 150ng mL^{-1} for water and 380 for feed. The main recovery is 99.4 ± 1.3%. The decision limit (CCα) is 26ng mL^{-1} for water and 190ng mL^{-1} for feed, detection capability (CCβ) is 30ng mL^{-1} and 217ng mL^{-1} for feed. Specificity, sensitivity and repeatability have also been validated using this protocol.

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