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METHOD FOR THE DETECTION OF DEXAMETHASONE IN CATTLE DRINKING WATER

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Introduction

Corticosteroids, such as dexamethasone, have many physiological roles. Dexamethasone (9-α-fluoro-16-α-methylprednisolone) is a synthetic glucocorticoid that is authorized for therapeutic use in veterinary medicine but its use as growth promoting agents 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 ilegally used to obtain an economical benefit through an increased muscle development. But these substances remain in meat and have negative toxic consequences for consumers. In the inspection control, drinking water and feed given to the animals may be sampled at the farm.

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 has been validated according to this Decision.

In this paper, a screening and confirmatory method for dexamethasone detection in drinking water in livestock has been developed and validated. This method is based on inmunoaffinity chromatography followed by reverse-phase high performance liquid chromatrography (IAC-RP-HPLC) with diode array detection at 242 nm.

Objectives

The goal of this work was the optimization and validation of a procedure for the detection of dexamethasone in water for cattle through inmunoaffinity chromatography followed by reverse-phase high performance liquid chromatography (RP-HPLC).

Methodology

The chemical structure of dexamethasone (9-α-fluoro-16-α-methylprednisolone) and flumethasone are shown in figure 1. A scheme of the extraction protocol is shown in figure 2. Briefly, 5 mL of drinking water with added internal standard

(flumethasone) were placed in a test tube. The solution was loaded into an immunoaffinity column, containing specific antibodies for dexamethasone, and washed with 10 mL of diluted buffer and 5 mL of mili-Q water. Corticosteroids were eluted with 4 mL of ethanol:water (70:30), pH 5.0, and collected into test tube that was evaporated to dryness at 45ºC under nitrogen stream. Then, the evaporated sample was resuspended in 200 µL of mobile phase consisting in acetonitrile:mili-Q water (30:70). 20 µL were injected into an Agilent series 1100 HPLC equipped with a diode-array detector. The column was a Synergi Max RP, 150 mm x 4.6 mm, from Phenomenex. The mobile phase, at a flow rate of 1 mL per min, was a solution consisting in acetonitrile:mili-Q water (30:70) and the eluent was monitored at 242 nm. In general, the following order of injections was followed for each set of samples: i) reagent blank, ii) compliant (water blank) control sample, iii) sample to be confirmed, iv) compliant control sample again and v) non-compliant (water fortified with 26 ng mL^{-1} dexamethasone) control sample.

Results & Discussion

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

Specificity: A total of 20 drinking water samples were analyzed. Other water samples were fortified with flumethasone and betametasone, individually and altogether. The method discriminated very well between the analyte (dexamethasone) and closely related substances as can be appreciated in the chromatogram (see figure 2). As can be observed, potentially interfering substances (chemically related compounds) eluted at different retention times.

Recovery: The recovery was determined by experiments using a total of 24 fortified blank water samples. Three sets, 8 samples each, were added dexamethasone to a final concentration of 26, 39 and 52 ng mL^{-1} for each set, respectively. The recoveries standard deviations and coefficients of variation were determined (see table 1). The mean recovery was 99.4 ± 1.3 % (mean ± SD).

Repeatability: A set of homogenized water blank samples were fortified with 26, 39 and 52 ng mL^{-1} of dexamethasone. At each level, the analysis was performed with 6 replicates and the mean concentrations, standard deviations and coefficients of variation were determined (see table 2).

Decision limit (CCα): 22 blank water samples were analyzed and the signal to noise ratio calculated in the time frame where dexamethasone is expected. The decision limit was set as 3 times the signal to noise ratio. This gives a CCα = 26 ng mL^{-1}.

Detection capability (CCß): 20 blank water samples were fortified with 26 ng mL^{-1} of dexamethasone, that corresponds to the decision limit, and the standard deviation was calculated. 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}.

Conclusions

The method based on immunoafinity chromatography followed by RP-HPLC for the analysis of dexamethasone in cattle drinking water has been validated using water fortified at levels up to 150 ng mL^{-1}. The main recovery is 99.4 ± 1.3%. The decision

limit (CCα) is 26 ng mL^{-1} and detection capability (CCβ) is 30 ng ml^{-1}. Specificity, sensitivity and repeteability have been also validated using this protocol.

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References

Cherlet M, De Baere S, De Backer P (2004) Quantitative determination of dexamethasone in bovine milk by liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry. Journal of Chromatography B, 805, 57–65.

Creaser CS, Feely SJ, Houghton E, Seymour M (1998) Immunoaffinity chromatography combined on-line with high-performance liquid chromatography-mass spectrometry for the determination of corticosteroids. Journal of Chromatography A, 794, 37–43.

Delahaut Ph, Jacquemin P, Colemonts Y, Dubois M, De Graeve J, Deluyker H (1997) Quantitative determination of synthetic corticosteroids by gas. Chromatography-mass spectrometry after purification by immunoaffinity chromatography. Journal of Chromatography B, 696, 203–215.

Draisci R, Marchiafava C, Palleschi L, Cammarata P, Cavalli S (2001) Accelerated solvent extraction and liquid chromatography-tandem mass spectrometry quantitation of corticosteroid residues in bovine liver. Journal of Chromatography B, 753, 217–223.

EC (2002) Commission Decision implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of the European Communities, 17.8.2002.

Stolker AAM, Schwillens PLWJ, Van Ginkel LA, Brinkman UATh (2000) Comparison of different liquid chromatography methods for the determination of corticosteroids in biological matrices. Journal of Chromatography A, 893, 55–67.

Tables and Figures

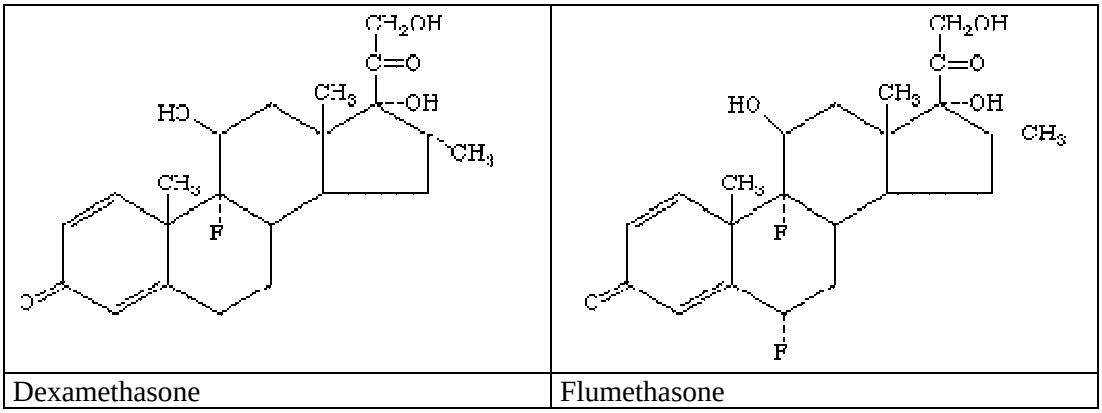
Table 1.- Recovery

Level (ng mL^{-1})	**Mean recovery (%)**	**Standard deviation**	**CV (%)**
26	105.1	1.73	6.33
39	98.5	1.08	2.80
52	94.5	1.09	2.22
Mean	99.4	1.30	3.78

Table 2.- Repeteability

Fortified level (ng mL^{-1})	**Mean concentration** (ng mL^{-1})	**Standard deviation**	**CV (%)**
26	30.4	0.70	2.32
39	45.5	0.61	1.34
52	63.0	2.42	3.84

Figure 1.- Structure of dexamethasone and flumethasone (internal standard)



Dexamethasone | Flumethasone

Figure 2.- Scheme of the extraction protocol

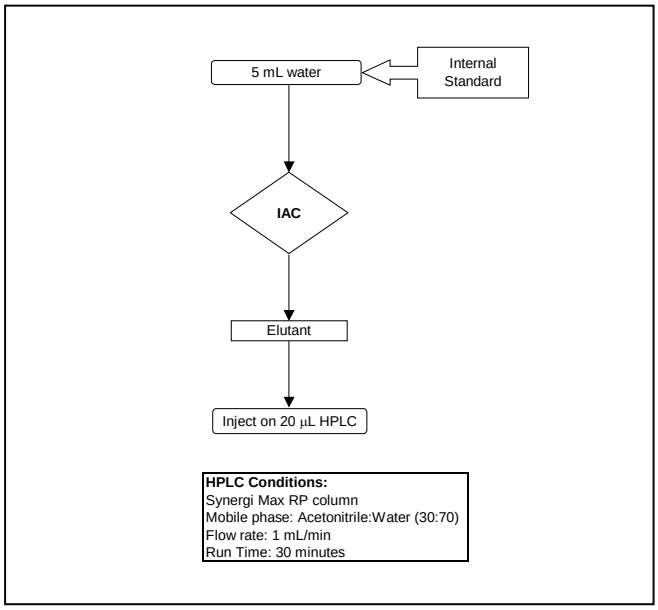


Figure 3.- Chromatogram of dexamethasone and closely related substances for the specificity study. Retention times of 10.14, 10.78 and 11.80 min were for betamethasone, dexamethasone and flumethasone, respectively.