Determination of Dexamethasone in Liver and Muscle by Liquid Chromatography and Gas Chromatography/Mass Spectrometry

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The rapid determination of dexamethasone (DEX) in bovine, porcine, and ovine liver and muscle has been accomplished by using a new extraction method. In liver, this procedure consists of adding sodium hydroxide to the tissue sample followed by extraction with ethyl acetate. After centrifugation, the organic supernatant underwent silica gel solid phase extraction. Muscle tissue can be analyzed by adding an acetonitrile/hexane partitioning step to this method. Analysis of the extracts is performed by using reversed-phase HPLC. A series of recovery curves performed at spiking levels of 5, 10, and 20 ppb have demonstrated that 70 and 60% of DEX can be recovered from liver and muscle tissue, respectively. A subsequent study indicated that this analytical procedure has a detection limit of 1.4 ppb. A variation of a previously published gas chromatographic/mass spectral (GC/MS) method for the analysis of dexamethasone in plasma has been used for the confirmation of DEX in extracts generated by the determinative method.

Keywords: *Dexamethasone; HPLC*

INTRODUCTION

Dexamethasone (9α-fluoro-11β,17α,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione, DEX; Figure 1) is a synthetic corticosteroid which stimulates parturition and glucogenesis and will "prevent or suppress the development of local heat, redness, swelling, and tenderness by which inflammation is recognized at the gross level of observation" (*The Pharmacological Basis of Therapeutics*). Use of this synthetic corticosteroid is limited to non-food-producing animals (*Code of Federal Regulations*); however, there is circumstantial evidence that DEX has sometimes been illegally used to assist swine, beef, and sheep in passing an antemortem inspection. A fast and sensitive method for the determination of DEX in bovine, porcine, and ovine liver and muscle tissue is therefore needed.

Good methods exist for analysis of this corticosteroid in human and animal serum (Alvernie and Toutain, 1982), urine (Derendorf et al., 1986), and bovine milk (de Paolis et al., 1977). Little work has been done on the analysis of DEX in animal tissue. Shearan et al. (1991) described a reversed-phase high-performance liquid chromatographic (HPLC) method for the determination of DEX in bovine muscle, liver, kidney, and fat tissues with a detection limit of 10 ppb. McLaughlin and Henion (1990) developed a coupled-column normal-phase HPLC method for the determination of DEX in bovine liver and muscle. Limits of detection for this latter method were estimated to be 4 and 6 ppb in beef muscle and liver, respectively.

This paper describes a reversed-phase HPLC/UV method for the determination of DEX in porcine, bovine, and ovine liver and muscle tissue that is more rapid and sensitive than any previously published method. A gas chromatographic/mass spectral method for the successful identification and confirmation of the presence of dexamethasone in extracts obtained from the determinative method is also described. A detection limit of 1.4 ppb was determined for the HPLC analytical method. Average recoveries of 70 and 60% were obtained in liver and muscle tissue, respectively, for both methods.

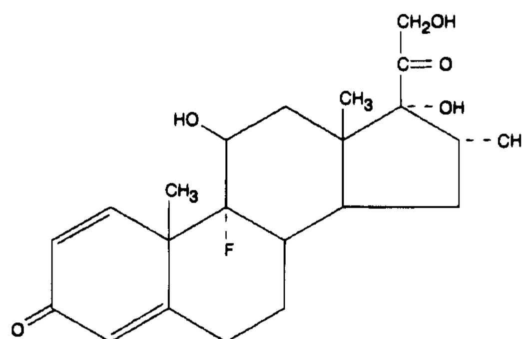

Figure 1. Structure of dexamethasone.

For liver, the determinative method consists of extraction of the analyte in ethyl acetate, which is a variation of that used by Shearan et al. (1991). This solvation step is followed by a silica gel solid-phase extraction and HPLC analysis. One analyst can prepare eight liver tissues for liquid chromatographic determination in 3 h. For muscle, an additional defatting step was necessary.

The confirmatory method follows that which has been described by Kayganich et al. (1990) with some changes in reagent amounts and reaction times. The derivatization is performed in 4 h. Using the GC/MS parameters described, DEX is readily detected in extracts from tissues containing 10 ppb of this synthetic corticosteroid.

METHOD

Apparatus. *Determinative Method.* All apparatus listed may be substituted with an equivalent if necessary: (a) silica gel SPE columns, Model 7086-03 (J. T. Baker Inc., Phillipsburg, NJ); (b) SPE vacuum manifold, Model 7018-00 (J. T. Baker); (c) SPE vacuum manifold stopcocks, Model 7241-00 (J. T. Baker); (d) liquid chromatograph, Hewlett-Packard Model 1050 with multiple wavelength detector and Model 3396 integrator (Hewlett-Packard Co., Rolling Meadows, IL); (e) LC

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column, Merck Hibar, Lichrosorb, RP-18 5 μm 4.0 × 250 mm (EM Science, Gibbstown, NJ); (f) LC precolumn, Model 933005 Brownlee C-18 3.2 × 15.0 mm (P. J. Cobert, St. Louis, MO).

Confirmatory Method: (a) Reacti-therm heating/stirring module, Model 18970 with block drilled to fit 16 mm test tubes with a depth of 20 mm (Pierce Chemical Co., Rockford, IL); (b) spin-bar, 10 mm × 3 mm (VWR Scientific, Media, PA); (c) Atmos-bag, polyethylene, Z11,283-6 (Aldrich Chemical Co., Milwaukee, WI); (d) Finnigan TSQ-70 operating in the NICI single quad mode (Finnigan MAT, San Jose, CA); (e) Varian Model 3400 gas chromatograph (Varian Associates, Inc., Walnut Creek, CA); (f) J&W DB-5 30 m × 0.25 mm i.d. with a 0.25 μm film thickness (J&W Scientific, Folsom CA).

Reagents and Materials. *Determinative Method.* (a) 20% acetone in hexane was used as the SPE column prewash solution. Add 20 mL of acetone to 80 mL of hexane. This solution is to be made up daily. (b) 40% acetone in hexane was used as the SPE column wash and elution solution. Add 40 mL of acetone to 60 mL of hexane. This solution is to be made up daily. (c) 0.02 M dibasic potassium phosphate buffer was prepared by adding 3.5 g of dibasic potassium phosphate to 1000 mL of deionized water. Adjust pH to 6.5 with concentrated phosphoric acid. (d) The mobile phase was 68% 0.02 M dibasic potassium phosphate, 32% acetonitrile. Add 680 mL of dibasic potassium phosphate buffer to 320 mL of acetonitrile. Pass through a 0.22 μm filter before use.

Confirmatory Method. (a) Celite, high purity, analytical grade, was obtained from Aldrich. (b) Pyridinium chlorochromate, 98% pure, was also obtained from Aldrich. (c) 4:1 Celite in pyridinium chlorochromate was prepared by grinding Celite with pyridinium chlorochromate in a mortar and pestle for 5 min under nitrogen in an Atmos-bag. This reagent is stored in a screw-top glass bottle that is protected from light. (d) Silica gel SPE columns, part no. 601303 were used (Analytichem International, Harbor City, CA). (e) 10% acetone in methylene chloride solution was prepared by adding 10 mL of acetone to 90 mL of methylene chloride.

Standards and Fortification Solutions: (a) dexamethasone (Sigma Chemical Co., St. Louis, MO); (b) DEX stock solution (1000 μg/mL) (add 25 mg of DEX to a 25 mL volumetric flask and dilute to volume with ethyl acetate); (c) DEX fortification solution (1 μg/mL or 1 ng/μL) (add 0.1 mL (or 100 μL) of the stock solution to a 100 mL volumetric flask and dilute to volume with ethyl acetate); (d) 10 ppb DEX external standard (using an Eppendorf pipet, transfer 50 μL of the fortification standard to a test tube and evaporate to dryness; reconstitute in 200 μL of mobile phase).

Tissue Homogenization and Extraction. *Liver. (1) Sample Homogenization.* Trim away fat and connective tissue and then place liver sample in a Waring blender. Homogenize the liver sample. Store in a freezer (temperature −15 °C) until the tissue is to be analyzed. LC chromatograms of samples stored for more than 3 weeks may contain more matrix components.

(2) Sample Extraction. Add 5 g of liver to a 50 mL centrifuge tube. Prepare a 10 ppb recovery by spiking a blank tissue sample with 50 μL of the 1 ng/μL fortification solution. Add 10 mL of the 0.3 M sodium hydroxide solution to each sample and shake for 2 s. Add 20 mL of ethyl acetate and shake for 1 min. Centrifuge these samples for 10 min at 2000 rpm (823*g*). Using a pipet, transfer the supernatant to another 50 mL centrifuge tube. Evaporate this solution to dryness in an N-Evap with a water bath temperature of 50 °C. Add 1 mL of ethyl acetate to the dried residue and vortex. Add 1 mL of hexane to the ethyl acetate and vortex again. Prepare the silica gel solid-phase extraction columns by adding 2 mL of 20% acetone in hexane. Allow this solution as well as all others to be drawn through the SPE columns under a vacuum of 1 in. of mercury. Add the samples to the SPE columns. Add 2.5 mL of 40% acetone in hexane to each test tube that contained a sample and wash each column with this solution. Discard the column wash solution. Add 3 mL of 40% acetone in hexane to each SPE column and collect the effluent. Evaporate the collected effluent to dryness, reconstitute in 200 μL of mobile phase, and vortex well. Filter the extract through a 0.22 μm Acrodisc into a 300 μL autosampler vial. Inject 50 μL of extract.

Muscle. (1) Sample Homogenization. Pass muscle tissue through a meat grinder once (plate size $^{5}/_{32}$ in.). Store in a freezer (temperature −15 °C) until the tissue is to be analyzed. No time limit for storage of muscle samples has been determined. Muscle tissues were always analyzed within 2 or 3 weeks of preparation.

(2) Sample Extraction. Proceed as described for the extraction of liver but after the ethyl acetate supernatant is evaporated to dryness, add 1 mL of acetonitrile to the dried residue and vortex. Add 3 mL of hexane to the acetonitrile and vortex for 10 s. Allow the layers to separate and remove the hexane layer. Evaporate the acetonitrile layer to dryness on an N-Evap with a water bath temperature of 50 °C. Add 1 mL of ethyl acetate to the dried residue and continue as described for the liver extraction.

LC Conditions. Operate the LC system with a flow rate of 1.0 mL/min of the mobile phase described under Reagents and Materials. The analytical column temperature controller is set at 35 °C. The total system back-pressure is 2100−2300 psi. Operate the UV detector at a wavelength of 239 nm with a bandwidth of 10 nm. If a Hewlett-Packard 1050 LC is being used with the multiwavelength detector, set the reference wavelength to 350 nm with a bandwidth of 80 nm. The integrator operates at a sensitivity of 2 mAu full-scale deflection (attenuation = 0 for the Hewlett-Packard Model 3396 integrator). The chart speed is set at 0.7 cm/min. A run time of 16 min was most frequently used.

Calculations. *Screening.* External standards where 6.25, 12.5, and 25 ng of DEX were injected on-column (equivalent to 5, 10, and 20 ppb) were analyzed prior to the injection of samples. Calculate the DEX content in parts per billion on manually measured peak heights as follows:

$$\text{ppb content in sample} = ((P_{samp}/P_{es}) \times N_{es} \times 4)/(R \times SW)$$

P_{samp} represents the peak height of DEX in the sample analyzed. P_{es} represents the peak height of the DEX external standard. N_{es} represents the number of nanograms of DEX injected on-column for the external standard. These terms are multiplied by 4 to represent the number of nanograms present in the autosampler vial if only one-fourth of the sample (50 of 200 μL) is injected. R represents the ratio of the peak height of DEX in a 10 ppb recovery versus that found for a 10 ppb external standard, assuming that the injection volume and attenuation were the same for both injections. SW is the weight of the sample prior to extraction.

Quantitation. Calculate quantitative values by generating a regression curve or response (in millimeter peak height) of the 5, 10, and 20 ppb of external standards versus the number of nanograms of DEX on-column. A linear curve described by the equation $Y = MX + B$ results, where Y represents the response value of the analyte in the sample, M represents the slope of the regression curve, X represents the number of nanograms of DEX on-column, and B represents the y-intercept of the regression curve. Solving the equation describing a linear curve for X yields $X = (Y - B)/M$. The value of X can then be used in place of $(P_{samp}/P_{es})(N_{es})$ in the equation shown under Screening. The factor described by $4/(R \times SW)$ is then multiplied by X to compute the level of DEX present in the tissue sample.

Preparation of 11,17-Diketo Derivative of Dexamethasone. Except for some changes in quantities of reagents and the reaction conditions used, the method described here is the same as that described by Kayganich et al. (1990): transfer the remaining buffered extract from the screening procedure to a 16 × 125 mm flat-bottom disposable screw-topped test tube and take to dryness at 50 °C under nitrogen; add 2.0 mL of methylene chloride, 20 mg of sodium acetate, 20 mg of a 1:4 mix of pyridinium chlorochromate and Celite, and a small stirring bar; cap, stir, and heat at 70 °C for 3 h such that the solution is below the top of the block. Refluxing should occur as 80% of the tube above the block acts as an air condenser. Poor yields of the derivative will occur if the solution does not reflux. Condition a silica SPE column by washing with 6 column volumes of methylene chloride under house vacuum;

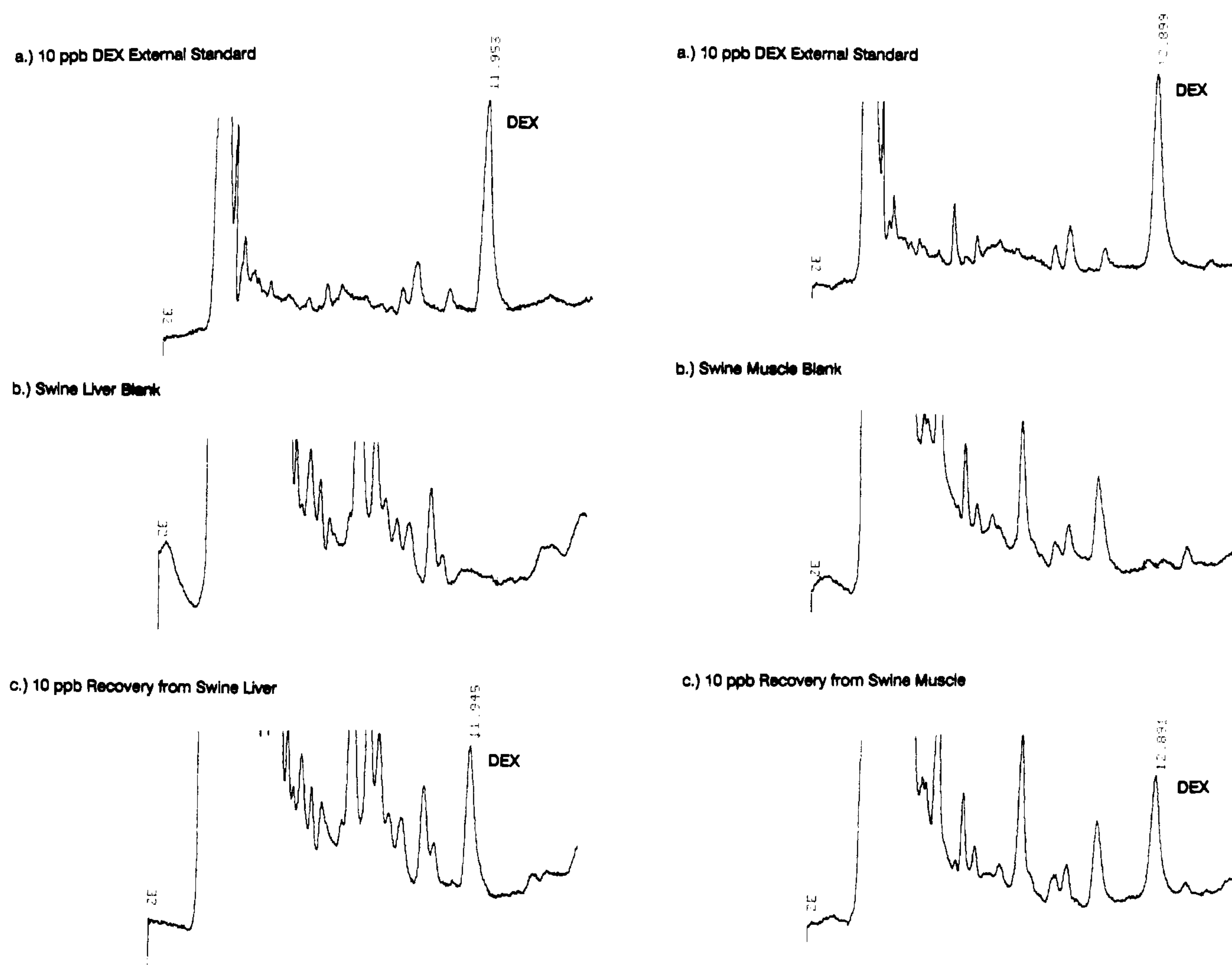

Figure 2. Typical chromatograms from swine liver (A, left) and muscle (B, right) tissues.

wash with 1 column volume at a drip rate of 2−4 drops/s using pressure from a 1 mL dropper bulb; do not allow columns to go dry; transfer the solution from the test tube to the column and allow to elute at 2−4 drops/s; collect eluate in a 15 mL disposable centrifuge tube. Elute the derivative with two 2 mL additions and then one 1 mL addition of 10% acetone in methylene chloride; evaporate the organic phase to dryness at 40 °C under nitrogen; dilute the residue to 1 mL with ethyl acetate. Any dilution from 100 μL to 1 mL may be used depending on the sensitivity of the instrument used. Inject from 1 to 3 μL into the system; monitor ions 295 (M − CH_3 − HF), 310 (M − HF), and 330 (M).

GC/MS Instrumentation and Operating Conditions. GC/MS analysis was done using a Varian 3400 gas chromatograph directly interfaced to a Finnigan TSQ-70 operating in the NICI single quad mode. The injection port temperature of the gas chromatograph was 260 °C with the transfer line set to 300 °C. After injection, the oven temperature was held at 100 °C for 1 min and then increased to 260 °C at 40 °C/min. The column temperature was increased to 300 °C at 4.0 °C/min and was held for 2 min. The reagent gas used for negative chemical ionization was methane. The source of the mass spectrometer was held at 140 °C, while the manifold temperature was maintained at 70 °C. The emission current at a potential of 70 eV was 0.20 mA. The multiplier was set at 900 V. A dwell time of 100 ms was used while *m/z* 295, 310, and 330 ions were monitored. An autotune in the NICI mode was performed prior to analyzing samples. A 5 ppb external standard was injected, and the source temperature was adjusted such that the ions at *m/z* 295 and 330 were nearly equivalent.

RESULTS AND DISCUSSION

Theory. The initial step of this extraction procedure disrupts the binding of albumin and α-globulin to DEX. The use of a 0.3 M sodium hydroxide solution in the initial extraction step prevented the introduction of partially soluble matrix components into subsequent extraction steps. The effectiveness of this partition was demonstrated by a comparison of ethyl acetate supernatants of liver samples extracted against 0.1 M NaOH versus those partitioned against 0.3 M NaOH. The latter supernatants were less highly colored than those extracted with a 0.1 M NaOH solution.

Initially, analyte elution off the SPE column was accomplished using methanol. A subsequent partition was required to remove other matrix components prior to HPLC analysis. Therefore, we changed the column wash and elution procedure, where 40% acetone in hexane was used for both SPE steps. This change in the column wash and elution steps caused fewer matrix components to elute from silica gel column while still efficiently recovering DEX. With this solid-phase extraction procedure no further cleanup was necessary.

As described under Method, an additional partitioning step is needed for the extraction of DEX from muscle tissue. When the extraction method for liver tissue was applied to spiked muscle tissue without modification, the total method recovery for DEX was less than 40%. Experimental results indicated that a 31−35% loss of analyte occurred during the silica gel SPE step. The

Table 1. Extraction Recovery of Dexamethasone from Swine, Beef, Veal, and Lamb Liver and Muscle

concn (ppb)	*n*	% recovery (mean ± SD)	% CV
swine liver			
5	12	71.3 ± 4.9	6.9
10	12	71.9 ± 5.4	7.4
20	12	74.7 ± 6.4	8.6
swine muscle			
5	12	56.1 ± 4.6	8.1
10	12	55.5 ± 4.4	7.8
20	12	58.6 ± 5.5	9.4
beef liver			
5	5	70.4 ± 5.1	7.2
10	5	69.6 ± 4.4	6.3
20	5	75.6 ± 3.4	4.4
beef muscle			
5	6	57.2 ±10.5	18.4
10	6	55.2 ± 7.3	13.1
20	6	59.5 ± 7.3	12.3
veal liver			
5	5	66.2 ± 3.1	4.7
10	5	71.0 ± 5.6	7.9
20	5	74.4 ± 3.4	4.5
veal muscle			
5	4	61.5 ±10.3	16.8
10	4	57.5 ± 6.0	10.4
20	4	61.0 ± 4.9	8.0
lamb liver			
5	3	68.3 ± 5.1	7.5
10	3	75.0 ± 3.0	4.0
20	3	79.0 ± 4.5	5.7
lamb muscle			
5	3	57.0 ± 8.1	14.4
10	3	61.7 ± 6.7	10.8
20	3	62.7 ± 4.9	7.9

Table 2. Dexamethasone Method Characterization: Results of Unknown Spiked Samples

sample	level fortified (ppb)	level found (ppb)	% recovery
	Swine Liver		
1	15	16	107
2	8	8.9	111
3	4	4.2	105
4	8	8.4	105
5	15	17	113
6	blank	blank	
7	30	31	103
	Swine Muscle		
1	6	6.1	102
2	28	31	111
3	blank	blank	
4	14	16	114
5	6	6.7	112
6	12	13	108
7	14	15	107

use of the acetonitrile/hexane partition immediately prior to the silica gel SPE increased the average recovery of DEX from muscle tissue from less than 40% to around 60%. Incorporation of the defatting step prior to solid-phase extraction prevented the introduction of large amounts of matrix components onto the silica gel stationary phase, maintaining the desired normal-phase extraction chemistry. Chromatograms generated by this extraction method for muscle and liver are shown in Figure 2.

Characterization. The initial characterization of this method involved the repeated generation of recovery curves for the liver and muscle of swine, beef, veal, and sheep at 0, 5, 10, and 20 ppb. At least two randomly selected tissue samples were used for each species/tissue combination. A minimum of three recovery curves was performed on different days for each of these combinations. Two analysts generated the recovery curve data

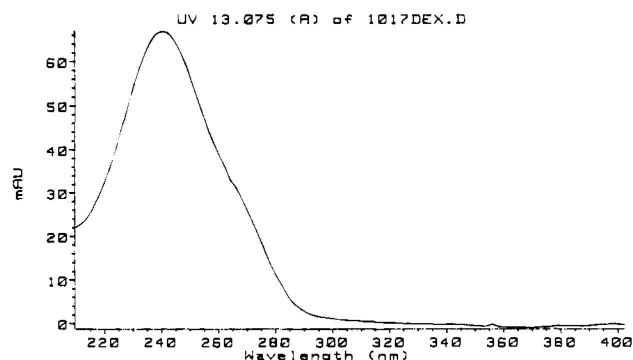

Figure 3. UV spectrum of dexamethasone taken by the HP 1040 diode array detector.

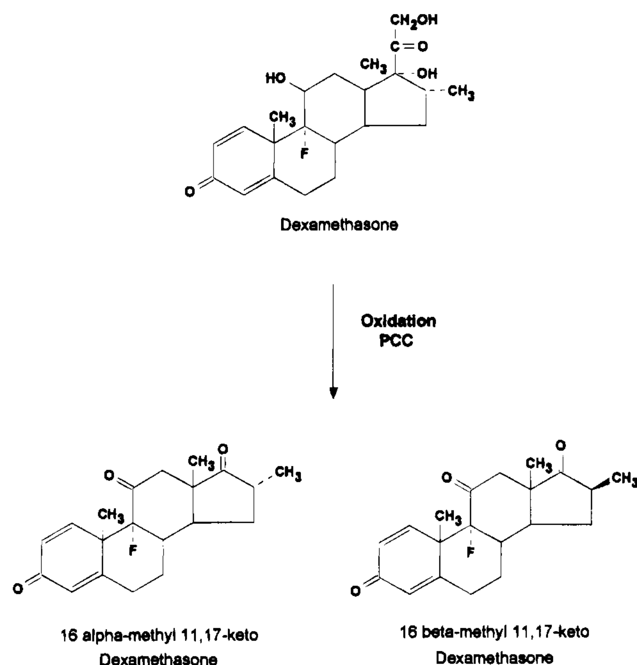

Figure 4. Conversion of dexamethasone to the 11,17-keto form by chemical oxidation with pyridinium chlorochromate.

for the liver and muscle of pork and beef. Recoveries were measured by peak height comparison to a 10 ppb external standard of DEX. Average liver recoveries over all species and spiking levels ranged from 66.2 to 79.2%. Average muscle recoveries over all species and spiking levels ranged from 55.2 to 62.7% (Table 1). The extraction of two of the 5 ppb spiked swine muscles and one 10 ppb spiked beef muscle recovered less than 50% of the analyte. None of the recovery curves performed in liver tissue contained any individual recovery that was below 60%. The coefficients of variation for any spiking level within a species/tissue combination were no greater than 18.4%. Higher coefficients of variation are apparent in muscle tissue. This increased variability is caused by the added manipulation of extracts of this tissue. The correlation coefficients for all recovery curves were greater than 0.9943 and were frequently above 0.9990.

A series of seven liver and seven muscle samples were spiked at unknown concentrations from 0 to 30 ppb. Tissue samples were always spiked at concentrations other than 5, 10, or 20 ppb. One blank was included with each set of seven muscle or liver unknowns. The variations of the results generated by the DEX analysis from the designated spiking concentration were less than 15% for liver and muscle tissue (Table 2). All of the concentrations found by this method are greater than the spiking concentration, indicating a small

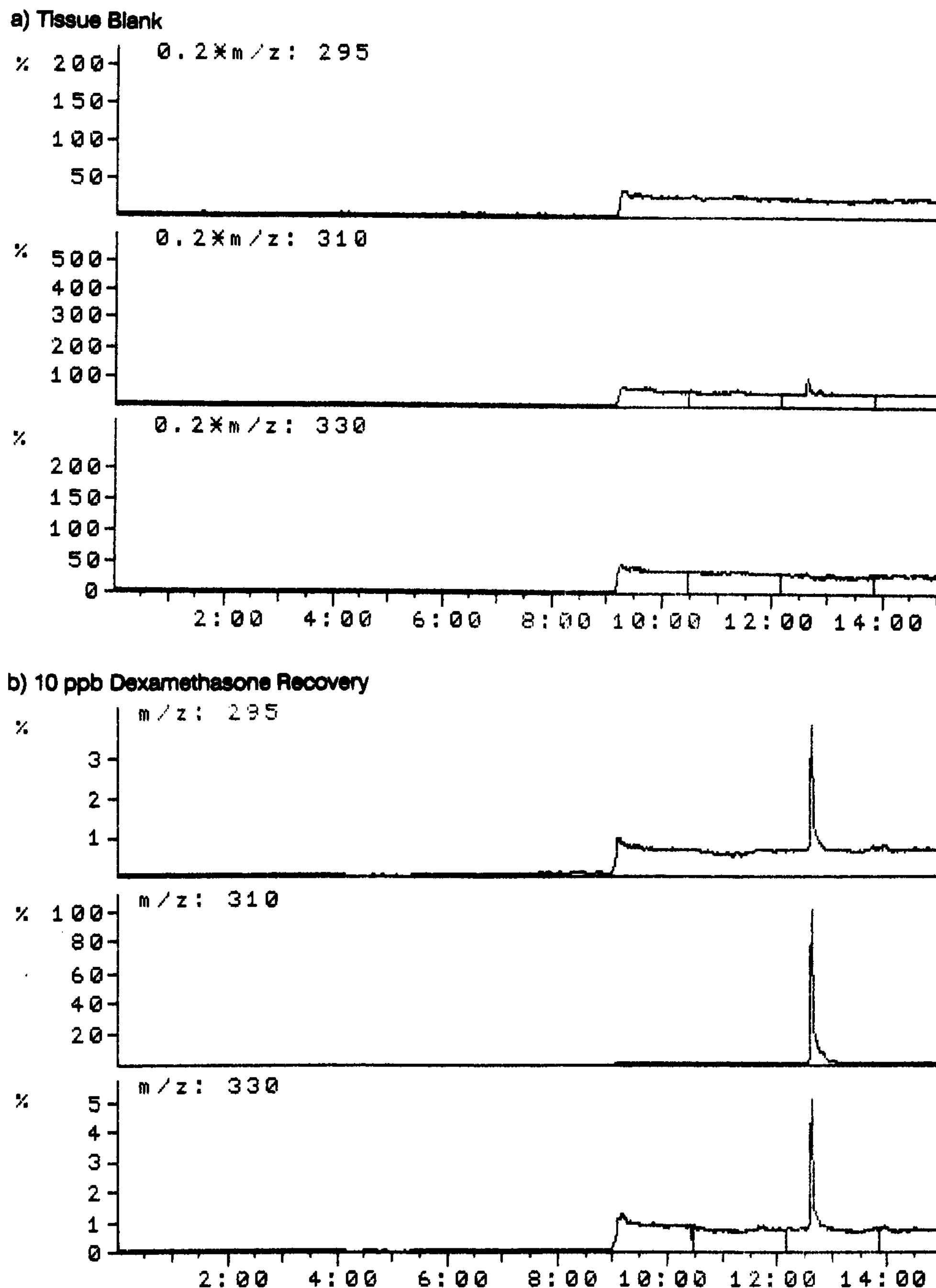

Figure 5. Selected ion chromatograms of a tissue blank and a 10 ppb dexamethasone recovery.

positive bias for this analysis. It has been determined that baseline irregularities generated by previous sample injections contribute to this positive bias. This can be significantly reduced by injecting the recovery at the end of sample set or by extending the run time such that no small peaks from previous runs elute near the retention time of DEX.

The efficacy of this method for the extraction of incurred DEX from liver tissue has also been verified. The method described here was compared with a procedure published by Shearan et al. (1991). The previously published extraction method has "been applied to studies on the persistence of dexamethasone in tissues following use of the drug in animal therapy." Both extraction methods demonstrated equivalent effectiveness in extracting incurred DEX from liver tissue. Using a beef liver from a previous dosing study, an average content of 44 ppb was found by the method described by Shearan et al. (1991). The analysis described here indicated that this beef liver contained, on average, 41 ppb of DEX.

Experimental results indicated that when 20 mL of ethyl acetate was used, 86% of the incurred DEX was solvated upon the first extraction. The remaining 14% was collected upon a second solvation. Only one extraction is performed in this method to increase the speed of this procedure. Speed is an important criteron for this determinative technique to be a practical method.

The *Official Journal of the European Community* (1989) (Annex to Council Directive 85/591/EEC, Section 1.27) defines the limit of detection (LOD) for an analytical method as "The smallest measured content from which it is possible to deduce the presence of the analyte with reasonable statistical certainty. It is equal to the mean of the measured content of representative blank samples ($N \geq 20$) plus 3 times the standard deviation of the mean". The FSIS accepts this definition for the LOD. On the basis of this guideline, the signal vari-

Table 3. Coefficients of Variation for Ion Ratios Found in the Validation Study of the Confirmation Method

day	species/tissue	% CV 295/310 ratio	% CV 330/310 ratio
1	beef liver	14	8
2	pork liver	11	16
3	veal and sheep liver	12	7
4	beef muscle	9	7
5	pork muscle	11	10
6	veal and sheep muscle	6	9
7	beef and pork liver	17	12
8	beef and pork muscle	11	15

ability over the retention time of DEX in 20 blanks was determined. The average signal deviation under the DEX peak was determined to be 2.1 mm with a standard deviation of 0.94 mm. Using the above criterion, the minimal peak height from which it would be possible to deduce the presence of analyte is 4.82 mm. This peak height would be generated by the presence of 1.4 ppb of DEX in a tissue sample. It should be emphasized that this detection limit is based on a calculated projection. The lowest spiking level that was detected and quantitated was 4.0 ppb (see Table 2).

Further Observations. Extracts generated by the DEX method have been injected on four different Merck Hibar RP-18 5 μm 4.6 × 250 mm analytical columns, with little variation in the resolution of DEX from matrix components. After using the same column for 2 months, the retention time of DEX had decreased from 12.5 to 10 min. No degradation in the ability of the analytical column to resolve DEX from other matrix components was noted.

The applicability of an HPLC system using a Waters 510 pump, a U6K injector, and a HP 1040 diode array detector has also been investigated. The same Merck C-18 5 μm analytical column was used without a precolumn. The temperature of this column was maintained at 35 °C with a column heater. The mobile phase consisted of 32% acetonitrile in water. Chromatograms of blank tissue extracts did not appear to contain any responses that would interfere with the quantitation of DEX in liver or muscle extracts. Using this HPLC system, the peak associated with DEX was broader due to the use of longer pieces of connective tubing. Additional confirmatory data can be obtained by a UV absorbance scan using a diode array detector (Figure 3).

GC/MS Confirmation of Dexamethasone. As described by Kayganich et al. (1990), the 11- and 17-hydroxy groups on DEX are oxidized to electrophilic carbonyls (Figure 4), yielding a product that can be easily detected in the concentration range of interest. Selected ion chromatograms of a 10 ppb recovery of DEX and a tissue blank from the same species are shown in Figure 5. Chromatographic resolution of the 16α- and 16β-methyl epimers of 11,17-ketodexamethasone was not accomplished. To test the performance of this confirmation procedure, a study was undertaken in which the presence of 10 and 20 ppb of DEX was verified in the extracts taken from the liver and muscle of beef, veal, swine, and sheep. No false negative or false positive results were found by the confirmation method for any of the animal tissue extracts. Statistical analysis of the intensity ratios of the m/z 295–310 ions and the m/z 330–310 ions indicated that an average coefficient of variation of 11% existed over all of the species/tissue combinations used in this study (Table 3). While this method was tested at 10 ppb, it should be able to confirm the presence of DEX to approximately 1 ppb. It should be noted that only three ions are monitored in this confirmation procedure. The mass spectral data are not the primary confirmation criteria used. Successful confirmation is based upon correct retention time in HPLC of the parent DEX as well as correct retention time of the oxidized DEX upon GC/MS analysis. The presence of the three ions in appropriate ratios further supports this identification. Together, these parts result in a very specific confirmation procedure.

CONCLUSIONS

It has been demonstrated that in a variety of species of liver and muscle this procedure can consistently recover around 70 or 60% of the DEX present in tissue, respectively. Using this extraction method, one analyst can prepare eight liver samples for HPLC analysis in 3 h. The speed of this analysis will permit the generation of chromatographic results for eight tissue samples on the same day that they are extracted. The detection limit study described here demonstrated that the presence of DEX in the liver or muscle of beef, swine, veal, or sheep can be detected to a level of 1.4 ppb. The gas chromatographic/mass spectral confirmation method described can verify the presence of DEX in extracts generated by the determinative method without further cleanup.

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