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Thin-layer chromatographic detection of zeranol and estradiol in fortified plasma and tissue extracts with Fast Corinth V☆

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ABSTRACT

In an attempt to improve sensitivity of thin-layer chromatographic (TLC) analysis and selectivity of visualizing agents for detection of estrogenic anabolic hormones, several dyes were screened for their chromogenic interactions with estrone, estradiol, diethylstilbestrol (DES), zeranol (zearalanol), zearalanone, and mycotoxins, zearalenone and zearalenol. Fast Corinth V salt was selected for its relatively high sensitivity. These anabolic compounds were separated by TLC and visualized with Corinth V and the results compared to iodine and starch visualization. Fortified bovine plasma and tissues (kidney, liver and muscle) and chicken muscles were analyzed after a clean-up procedure using solid-phase dual columns of alumina and anion-exchange resin. Iodine–starch clearly detected 4 ng of estradiol and DES while zeranol and zearalenone were detected at higher levels (10 ng). Fast Corinth V showed distinct spots with 2 ng of zeranol and 4 ng of zearalenone while faint spots were observed with estradiol and estrone standards. DES was not detectable at these levels. Less background interference was observed with Corinth V than with iodine–starch. The former confirmed spots detected by iodine–starch. This study suggests its selectivity for detection of zeranol and its metabolite, zearalanone, in the presence of steroidal compounds.

INTRODUCTION

Anabolic hormones are given to cattle and sheep to promote rapid growth and enhance feed efficiency. As a result, the animals can efficiently metabolize nitrogen from feeds for rapid protein conversion. These manipulations result in economic savings to the farmers and the consumers as well. Residues from these hormonal drugs (sanctioned by the Food and Drug Administration) pose no danger to the health of the consumers if United States government regulatory guidelines are adhered to and proper animal husbandry is practiced (CFR 1991). Abuse or misuse of veterinary drugs, in general, can leave illegal residues in edible tissues of treated animals. With data gathered from its 1989 monitoring programs nationwide, the Food Safety and Inspection Service of the US Department of Agriculture reported 99.7% adherence to these regulations [1]. Allowable residue levels [2] of veterinary estrogenic growth promotors are shown in Table I. Cereal grains used as animal feeds can be contaminated with zearalenone and zearalenol, mycotoxins produced by *Fusarium* molds [3–5].

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TABLE I

TOLERANCE LEVELS OF ANABOLIC HORMONES WITH ESTROGENIC ACTIVITY

These tolerance levels are expressed as concentrations of the free compounds and not the esters as indicated in the left column. Reference: *Code of Federal Regulations*, 1990. Diethylstilbestrol (DES) is banned for use in all food producing animals.

Compound	Animals	Tolerances[a]
Estradiol benzoate	Heifers, steers, calves	120 ppt M
		480 ppt F
		360 ppt K
		240 ppt L
	Lambs	120 ppt M
		600 ppt F, K, L
Estradiol monopalmitate	Chickens	0[b] (bioassay)
Zeranol	Cattle	150 ppb M
		300 ppb L
		450 ppb K
		600 ppb F
	Sheep	0 (GC–FID)[c]

[a] Uncooked edible tissues: M = muscle, F = fat, K = kidney, L = liver.
[b] Bioassay has a minimum detectability in ppm levels.
[c] Gas chromatography–flame ionization detection has minimum detectability of 25 ppb.

These contaminants, if in excessive amounts, can leave residual mycotoxin in the edible tissues of animal foods.

In our previous analytical studies [6,7] we have found that false positive results can occur if these mycotoxins (zearalenone and its metabolite) are not resolved from the drug residues, zeranol and its metabolite, zearalanone.

In a limited survey of beef liver, Roybal *et al.* [8] showed no detectable amounts of zearalenone at < 1 ppb. However, zearalenone was reportedly detected in animal products, mostly milk and poultry muscle, from animals fed high levels of zearalenone. These data are summarized by Kuiper-Goodman *et al.* [3]. "Incurred" levels of zearalenone in animal products have not been reported due to lack of adequate rapid screening methods capable of detecting levels that could be present in tissues of animals consuming feed contaminated with zearalenone at levels normally encountered in nature [9].

Numerous instrumental methods have been reported for analysis of zeranol, estradiol and other anabolic hormones. These methods offer a wide spectrum of analysis, but are tedious and expensive requiring technical skills. Relevant techniques were briefly reviewed in our previous publications [6,7]. In addition, Covey and co-workers [10,11] and Maffei Facino *et al.* [12] described a gas chromatographic–mass spectrometric (GC–MS) and high-performance liquid chromatographic–tandem mass spectrometric (HPLC–MS–MS) analysis of zeranol and other anabolics for detection and confirmation at low ppb. Roybal *et al.* [8] reported the analysis of zeranol and zearalenone and Fukuhara *et al.* [13] detected zeranol and estradiol with HPLC using electrochemical detection and confirmed results with MS. HPLC separation with ultraviolet detection of zeranol and other estrogenic anabolic hormones were also developed [6,14]. Radioimmunoassays were used to measure zeranol and metabolites after solvent extraction of animal tissues and fluids [15,16]. Antibodies for estrogenic compounds showed high cross-reactivities with metabolites and also had significant non-specific binding contributing to high background signals. Therefore, HPLC fractionation of zeranol from its metabolites or other anabolics was necessary prior to application of immunoassays in order to

obtain specific measurements of the analytes [17–19]. Batch immunoaffinity chromatographic clean-up was utilized for GC detection of zeranol and metabolites [20]. On-line HPLC immunoaffinity clean-up was described by Van Ginkel [21] for multi-residue detection of anabolic hormones.

Thin-layer chromatographic (TLC) methods are rapid, inexpensive and suitable for screening compounds prior to their analysis with other instrumental methods. Multi-residue TLC analyses of estrogenic growth promoters were reported by Gunther [22], Wortberg *et al.* [24], De Ruig *et al.* [25], Medina and Schwartz [7] and Van Look *et al.* [26]. The use of Fast Dark Blue R-salt, a diazonium dye, had been reported [22,23] for detection of estrogen-like compounds in food products. Wortberg *et al.* [23] reported minimum detection of estradiol and zeranol at 2 ng and a 50% recovery of zeranol in meat sample fortified at 0.8 ppm. Indeed, a more rapid and sensitive TLC method is still needed to screen the presence of these residues and to confirm their presence. The objectives of this study were to screen various dyes and select those giving stable chromogenic complexes to improve the visualization of anabolic estrogens and compare their sensitivity with the iodine–starch visualizing technique. The latter was observed in our laboratory to be more sensitive than fluorescent visualizing methods [7,24]. An improved TLC analysis of anabolic estrogens (estradiol and zeranol) isolated with the solid-phase technique [27] from animal plasma and tissue extracts is presented.

EXPERIMENTAL

Reagents and equipment

Acetone, hexane, methylene chloride, methanol and 2-propanol (all glass-distilled) were obtained from Burdick and Jackson (Muskegon, MI, USA); basic alumina (80–200 mesh, Brockman Activity I), iodine, toluene, sea sand, and widemouth bottle (4 oz) with screw cap from Fisher Scientific (King of Prussia, PA, USA); Ag-MP-1 ion-exchange resin and Poly-Prep disposable columns from Bio-Rad (Richmond, CA, USA; soluble starch from J. T. Baker (Phillipsburg, NJ, USA); dimethyldichlorosilane, 17β-estradiol, estrone, zearalenol, zearalenone, Fast Corinth V and other diazonium dyes (Table II) from Sigma (St. Louis, MO, USA) and Aldrich (Milwaukee, WI, USA); acetic acid (gold label) and diethystilbestrol from Aldrich; zeranol and zearalanone as gifts from International Minerals and Chemicals Corporation (Terre Haute, IN, USA); [^{3}H]-17β-estradiol from New England Nuclear (Albany, MA, USA); polypropylene tips (5 ml) from Rainin Instrument (Woburn, MA, USA); HPTLC-GHL silica gel plates (2.5 cm × 10 cm), from Analtech (Newark, DE, USA); Polytron homogenizer from Brinkman (Long Island, NY, USA); sonicator from Heat Systems (Long Island, NY, USA); and Centra R7 centrifuge from International Equipment (Needham, MA, USA).

Sample extraction

Chicken muscle, bovine skeletal muscle tissues and kidney were homogenized in a Waring blender and stored at −20°C. Extraction procedures for bovine tissues and fluids were followed as described in our previous work [27]. Aliquots of 1–2.5 g were extracted by homogenizing in 5 ml acetone–water (95.5, v/v) using the Polytron homogenizer for 2 min. The Polytron tip was rinsed with another 5 ml of solvent, and sample was sonicated further for 5 min using 75% pulsed power. Bovine fat and liver were extracted with sonication for 5 min. Samples were then centrifuged for 10 min at 3200 *g* (5000 rpm). The supernatant phases were decanted and the pellet was put aside and extracted with another 5 ml of solvent, sonicated for 5 min and centrifuged. The supernatants of each tissue were pooled and the volume was adjusted to 10 ml. Bovine plasma or serum (1 ml) was extracted with 8 ml of solvent by mixing vigorously with a vortex mixer or IKA-Vibrax shaker. The extract was separated by centrifugation at 3020 *g* for 10 min and applied to clean-up columns.

Extracts of bovine plasma and tissue were fortified with 125, 200 and 250 ppb of zeranol or estradiol as single compounds or binary mixture.

Chicken muscle extracts were fortified with 50 and 100 ppb of zeranol or estradiol and their binary mixture. The sample size and fortification levels are shown in Tables IV and V.

Solid-phase clean-up

Extracts containing fortified estrogens were isolated through solid-phase clean-up columns as we have previously described [27]. Aliquots of 5 ml were taken from each tissue extract, 2.5 ml of liver extract and 8 ml of plasma extract were applied to a 1.5-g basic alumina column (packed in a 5-ml pipet tip) placed in tandem with the ion-exchange column which contained 4 ml of 30% Ag-MP-1 resin suspension in the phosphate form [27]. Basic alumina was packed in a 5-ml disposable pipet tip plugged with a glass bead and 150 mg of sea sand to a bed volume of 15 mm × 12 mm I.D. Ag-MP-1 was packed in a Poly-Prep column to a bed volume of 37 mm × 10 mm I.D. The extracts were allowed to percolate by gravity from the alumina column into the ion-exchange column. After washing the alumina column with four 1-ml volumes of acetone–water (95:5, v/v), the alumina column was removed and the wall of the ion-exchange column was rinsed with 1 ml of acetone–water solvent. The estrogens were then eluted with four 1-ml portions of acetic acid–acetone (10:90, v/v), collecting the effluents in silanized vials. Eluates were dried under a stream of nitrogen. Water-soluble contaminants were extracted by mixing vigorously with 0.5 ml of distilled water. The estrogens were then extracted twice with 2 ml of diethyl ether by using an IKA shaker for 5 min. The aqueous phase was quick frozen in a dry ice–acetone mixture. The ether extracts were decanted into silanized vials and dried with a stream of nitrogen. The residues

TABLE II

COMMON AND CHEMICAL NAMES OF DYES USED IN THIS STUDY

Dye	Color index	Azoic diazo No.	Chemical name
1. Fast Black K Salt	37190	38	2,5-Dimethoxy-4-N-[(4-nitrophenyl)azo]benzenediazonium chloride hemi[zinc chloride] salt
2. Fast Blue B Salt	37235	48	3,3-Dimethoxybenzidine [zinc chloride] salt
3. Fast Blue BB Salt	37175	20	4-Benzoylamino-2,5-diethoxybenzenediazonium chloride hemi[zinc chloride] salt
4. Fast Blue RR Salt	37155	24	4-Benzoylamino-2,5-dimethoxybenzenediazonium chloride hemi[zinc chloride] salt
5. Fast Corinth V Salt	37220	39	4-Methyl-6-nitro-4-[(3-methyl-6-methoxyphenyl)azo]benzenediazonium hemi[zinc chloride] salt
6. Fast Garnet GBC Salt	37210	4	2-Methyl-4-[(2-methylphenyl)azo]benzenediazonium salt
7. Fast Red Al Salt	37275	36	Anthraquinone-1-diazonium hemi[zinc chloride] salt
8. Fast Red B Salt	37125	5	2-Methoxy-4-nitrobenzenediazonium salt
9. Fast Red KL Salt			2-Carbamoyl-5-methoxybenzenediazonium chloride hemi[zinc chloride] salt
10. Fast Red PDC Salt	37151	14	5-Butylamino-sulfonyl-2-methoxybenzenediazonium chloride hemi[zinc chloride] salt
11. Fast Red RC Salt	37120	10	5-Chloro-2-methoxybenzenediazonium chloride hemi[zinc chloride] salt
12. Fast Red TR Salt	37085	11	4-Chloro-2-methylbenzenediazonium hemi[zin chloride] salt
13. Fast Red Violet LB Salt			5-Chloro-4-benzamido-2-methylbenzenediazonium chloride hemi[zinc chloride] salt
14. Fast Violet B Salt	37165	41	4-Benzoylamino-2-methoxy-5-methylbenzenediazonium chloride hemi[zinc chloride] salt
15. Fast Yellow GC Salt	37000	44	2-Chlorobenzenediazonium [zinc chloride] salt

were reconstituted with 25–100 μl of hexane–methanol–2-propanol (80:15:5, v/v). All analyses were carried out in duplicate. Purification efficiency of the alumina ion-exchange columns was determined by measuring the retention and elution of 30 000 cpm of [^{3}H]estradiol placed in separate columns using identical batches of activated resin used in the clean-up of plasma and tissue extracts. Radioactivity of the tracer ([^{3}H]estradiol) in the flow through and eluted fractions were determined.

Screening of dyes

Fifteen dyes (Table II) were screened for the chromogenic reactions with estrone, estradiol, zeranol, zearalenone, zearalanone, zearalenol and diethylstilbestrol. The estrogens were applied to TLC plates at 10–40 ng/μl and sprayed with 0.025 or 0.05% aqueous dye solution. The TLC plates were exposed to ammonia or HCl vapors prior to or after spraying with the dye solution and subsequently placed in a 85°C oven or dried by a hair dryer. Optimum conditions were selected for subsequent TLC analysis of isolates of plasma and tissue samples.

Preparation of standards

Stock solutions were made to contain 1 mg/ml estrogen in methanol and stored below 0°C in vials treated with 2% dimethyldichlorosilane in toluene. Working dilutions of 2 or 10 μg/ml were prepared with hexane–methanol–2-propanol (80:15:5).

TLC analysis

A previously described TLC procedure [7] was followed. Wide mouth jars (112 ml or 4 oz) with screw caps were used as developing tanks. A 10-ml volume of developing solvent (methylene chloride–methanol–2-propanol, 97:1:2, v/v) was placed in jars and allowed to equilibrate for 15 min. TLC plates were washed with 50:50 (v/v) methanol–methylene chloride and activated at 110°C for 20 min prior to application of samples. Samples were applied at a volume of 2 μl containing a total anabolic estrogen concentration ranging from 1 to 10 ng/μl (Tables III, IV and V). The sample spots were dried and kept compact with a hair dryer while applying samples prior to chromatography. The plates then were equilibrated (for 15 min at 4°C with the solvent) by placing the plate on the dry part of a tilted jar. The jar was then placed upright in a 4°C refrigerator for development (6 min). After development, the plate was dried in an 85°C oven for 5 min and exposed to iodine vapor for 3–5 min. The background iodine was allowed to volatilize for 30 s, and spots were revealed by spraying with 1% aquous gelatinized starch solution prepared by heating the starch suspension above 70°C. The starch solution was allowed to cool down before using as a TLC spray. Alternatively, the developed plates were dried with a hair dryer for 5 min and sprayed with 0.05% aqueous solution of Fast Corinth V. The TLC plate was then placed in a 112-ml jar saturated with ammonia vapor and exposed for 30 s. The plates were further dried with a hair dryer until chromogens appeared.

RESULTS AND DISCUSSION

Table III shows a summary of interactions of fifteen dyes with the anabolic estrogens and mycotoxins. Diazonium dyes form diazo complexes with the phenolic and resorcylic groups of these estrogens to give chromatograms of varying color intensity. Free phenol is substituted at the *para*-position to the hydroxyl. However, these are blocked in the phenolic estrogens (estradiol, estrone or DES) and substitution or coupling of the diazonium dye is then favored at the *ortho*-position. Resorcylic compounds like zeranol and zearalenone are coupled at the 13-position, equivalent to the 4-position of resorcinol. A typical reaction of zeranol and Fast Corinth V is shown in Fig. 1. Fast Blue BB and Fast Corinth V had the strongest color intensity formed. Zeranol, zearalenone, zearalanone and zearalenol reacted more strongly than the phenolic compounds estrone, estradiol and DES. These results indicate that diazonium dyes can be used selectively for zeranol, its derivative and related mycotoxins. Wortberg *et al.* [23] also reported the higher sensitivity of Fast Dark Blue R with zeranol as with estradiol, estriol and estrone.

TABLE III

COLOR REACTION[a] OF DIAZONIUM DYES WITH ESTROGENIC GROWTH PROMOTERS[b] AND MYCOTOXINS[c]

Visualization: TLC plates spotted with estrogens were exposed to NH_3 vapor for 10 min, sprayed with 0.05% diazonium dyes in distilled water, dried in a 85°C oven (½ h) and sprayed with 1% sodium carbonate solution.

Dye	Color	E_1	E_2	Z_1	Z_2	Za_1	Za_2	DES
Fast Black K Salt	Purple (20 ng)	vf	vf	m	m	m	m	vf
Fast Blue B Salt	Purple (30 ng)	N	N	m	m	m	m	N.D.
Fast Blue BB Salt	Purple (20 ng)	N	N	m	d	d	d	N.D.
Fast Blue RR Salt	Purple (20 ng)	N	N	f	l	l	l	N.D.
Fast Corinth V Salt	Orange–red (30 ng)	f l (40 ng)	f l (40 ng)	md m (10 ng)	md m (10 ng)	md m (10 mg)	nd m (10 ng)	f m (40 ng)
Fast Garnet GBC	No color (30 ng)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Fast Red Al Salt	Yellow (30 ng)	N.D.	l	m	l	l	m	N.D.
Fast Red B Salt	Yellow (20 ng)	N.D.	N.D.	l	l	l	l	N.D.
Fast Red RL	Yellow (20 ng)	N.D.	N.D.	f	l	l	l	N.D.
Fast Red PDC Salt	Yellow (30 ng)	f	f	l	l	l	l	vf
Fast Red RC	Yellow (30 ng)	vvf	vvf	l	f	f	l	vvf
Fast Red TR Salt	No color (30 ng)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Fast Red Violet LB Salt	Orange (20 ng)	f	f	l	l	m	m	f
Fast Violet B Salt	Orange (20 ng)	N.D.	N.D.	l	l	l	l	N.D.
Fast Yellow GC Salt	Yellow (20 ng)	N.D.	N.D.	l	l	m	m	N.D.

[a] vvf = very, very faint; vf = very faint; f = faint; l = light; m = medium; d = dark; N.D. = not detectable.
[b] E_1 = estrone; E_2 = estradiol; Za_1 = zearalanone; Za_2 = zeranol (zearalanol); DES = diethylstilbestrol.
[c] Z_1 = zearalenone; Z_2 = zearalenol.

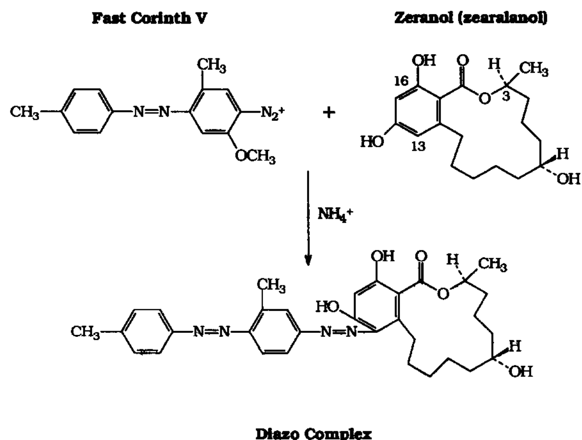

Fig.1. Diazo complex formation of Fast Corinth V with zeranol.

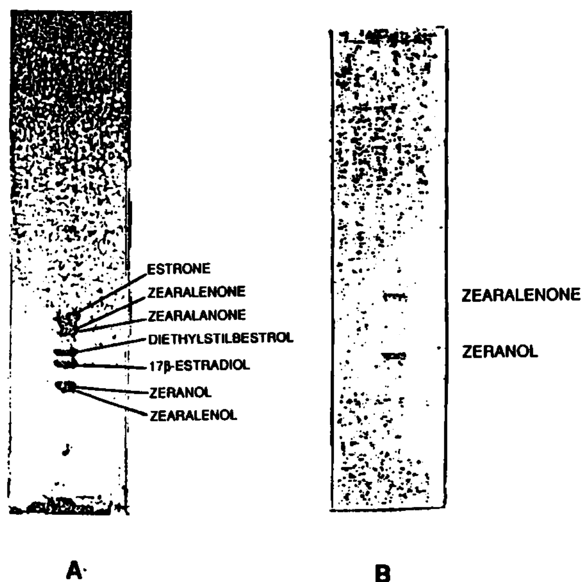

Fig. 2. TLC separation of estrogens developed in methylene chloride–methanol–2-propanol (97:1:2, v/v) and visualized with (A) iodine–starch, 50 ng each, and (B) Fast Corinth V, 25 ng each.

Multi-residue separation of seven anabolic estrogens analyzed at 10 or 20 ng showed five bands as was previously reported by Medina and Schwartz [7]. This study again shows the separation of these compounds into five bands (Fig. 2A and Table IV). These compounds were visualized with iodine–starch and Fast Corinth V and results show better detectability of zeranol and zearalenone with Fast Corinth V. Fig. 2B also shows the separation of zeranol and zearalenone. DES, estradiol and estrone were strongly detected with iodine–starch. DES was not detectable at 10 ng with Corinth V but was detectable with iodine–starch. Zearalenol and zearalanone, metabolites

TABLE IV

R_F VALUES OF SEVEN ESTROGENIC COMPOUNDS AND GROWTH PROMOTERS SEPARATED AS MULTI-COMPONENT

Compound	R_F			
	Iodine–starch		Fast Corinth V	
	10 ng	20 ng	10 ng	20 ng
Estrone (E_1)	0.21	0.22	0.25, 0.27 (O)	0.27 (O)[a]
Zearalenone (Z_1)	0.17	0.19	0.22, 0.20 (R)	0.24 (R)[a]
Zearalanone (Za_1)	0.17	0.19	0.22, 0.20 (R)	0.24 (R)
Estradiol (E_2)	0.15	0.15	0.19, 0.16 (O)	0.19 (O)
Diethylstilbesterol	0.11	0.12	N.D.[b], 0.14 (O)	N.D.
Zeranol (Za_2)	0.07	0.06	0.11, 0.12 (R)	0.14 (R)
Zearalenol (Z_1)	0.07	0.06	0.11, 0.12 (R)	0.14 (R)

[a] O = orange; R = orange–red.
[b] N.D. = not detectable.

TABLE V

DETECTION OF ZERANOL AND ESTRADIOL CHICKEN MUSCLE EXTRACTS

Sample size (g)	Added[a] (ppb)	Amount (ng)	R_F	
			Corinth V	Iodine–starch
2.5	100 Za_2	10	0.22	0.22
	100 E_2	10	0.26	0.25
2.5	100 Za_2	4	0.16	N.D.[b]
	100 E_2	4	N.D.	0.24
2.5	100 Za_2	4	0.16	N.D.
2.5	100 E_2	4	N.D.	0.24
2.5	50 Za_2	2	N.D.	N.D.

[a] Za_2 = zeranol; E_2 = estradiol.
[b] N.D. = not detectable.

of zearalenone (mycotoxin) and zeranol, respectively, could not be separated by TLC. In such a case, TLC results showing both zeranol and zearalenone spots must be confirmed by use of other methods such as HPLC. However, there is no known or reported "incurred" levels of zearalenone in poultry or bovine tissues [8]. As we have previously reported [7], these visualization techniques showed higher sensitivity than the fluorescent technique reported by Verbeke [24].

Chicken muscle

Table V shows the fortification levels of chicken muscle extract and their TLC results. The fortified extracts were purified through alumina ion-exchange dual columns and the eluates were re-extracted with water followed with diethyl ether extraction prior to TLC analysis. Results showed clear positive spots when 10 ng of zeranol and estradiol fortified at 100 ppb were visualized with Corinth V or iodine–starch. The minimum

TABLE VI

DETECTION OF ZERANOL AND ESTRADIOL FORTIFIED BOVINE PLASMA AND TISSUE EXTRACTS

Zeranol (Za_2) and estradiol (E_2) were added as binary mixtures. Results were confirmed with standards and compared with unfortified samples which were analyzed on the same plate with fortified samples (vf = very faint spot; f = faint spot; N.D. = not detectable).

Sample	Sample size	Added (ppb)	TLC-analyzed (ng)	R_F	
				Corinth V	Iodine–starch
Muscle	2 g	125 Za_2	10	0.20	0.19 (vf)
		125 E_2	10	0.25 (vf)	0.25
Liver	1 g	250 Za_2	5	0.21	N.D.
		250 E_2	5	N.D.	0.21
Kidney	1.25 g	200 Za_2	10	0.20	0.20
		200 E_2	10	0.25	0.25
Plasma	1 ml	125 Za_2	10	0.20	0.20 (f)
		125 E_2	10	N.D.	0.25

amount of detectable zeranol was at 4 ng with Corinth V but was not detectable with iodine–starch. In contrast, estradiol was not detected with Corinth V but was detectable at 4 ng with iodine–starch. Experiments with labeled estradiol showed that the sample clean-up procedure gave recoveries of 73 ± 5% (n = 16).

Bovine tissues and plasma

Fortification levels of zeranol and estradiol in bovine tissue extracts and plasma are shown in Table VI with results of their TLC analysis. Extraction and clean-up procedure were followed as described in previous paragraphs. Bovine muscle extract (2 g) was fortified at 125 ppb. Positive spots were detected with iodine–starch at 10 ng equiv. of zeranol (R_F = 0.20) and 10 ng of estradiol (R_F = 0.25) was detected with Corinth V. Extraction of clean-up column eluates with diethyl ether alone or water–diethyl ether showed identical TLC results. Similarly, extract from 1 g liver sample was fortified with 250 ppb of zeranol and estradiol, applying a quarter of the total volume to clean-up columns. The dried eluates were extracted with diethyl ether alone or water–diethyl ether. An equivalent of 5 ng zeranol (R_F = 0.21) was detectable with Corinth V after diethyl ether or water–diethyl ether extractions. Estradiol was not detected with iodine–starch or Corinth V at 5 ng. Interfering background spots resulted after iodine–starch visualization of diethyl ether extracts. Kidney samples (1.25 g) fortified with 200 ppb of zeranol and estradiol showed detectability at 10 ng when visualized with iodine–starch or Corinth V. The unfortified tissue extracts had no interfering spots after eluates were sequentially extracted with water and diethyl ether, then visualized with either Corinth V or iodine–starch. Likewise, in fortified plasma at 125 ppb, 10 ng of zeranol was detectable with Corinth V and iodine–starch, 10 ng of estradiol was detectable with iodine–starch. In all cases, the post-column extraction of the eluates with water–diethyl ether resulted in much cleaner samples (no interfering spots with estrogens) than extraction with diethyl ether alone. Clean-up recoveries of [^{3}H]estradiol were 62.2 ± 0.4% (n = 4), 76.4 ± 2.1% (n = 4) and 64.5 ± 7.3% (n = 4) when bovine muscle, liver and kidney extracts were purified, respectively, through the dual columns. The sequential water–diethyl ether extraction of the column eluates also yielded higher recoveries than extraction with diethyl ether alone. Hexane extractions produced negative results.

CONCLUSIONS

The extraction, purification and TLC procedures described provide a rapid, simple and inexpensive method to screen for low nanogram levels of zeranol, estradiol, DES and zearalenone. Results from this study show better resolution and detectability than those reported for TLC analysis of estrogen growth promoters. Fast Corinth V showed the highest sensitivity when used with solid-phase purified tissue and plasma samples and was selected as the visualizing agent from fifteen dyes. Fast Corinth V also showed selectivity for zeranol and related metabolites including mycotoxins at low nanogram levels. The stability of the diazo complex formed with Fast Corinth V provides us with a visualizing agent which can be used for quantitative TLC detection of zeranol at low ppb. Phenolic estrogens (estradiol, DES, etc.) had higher sensitive response to iodine and starch. Zeranol fortified in plasma and tissue extracts was detectable at 4 and 10 ng in 100-ppb samples when visualized with Corinth V and iodine–starch, respectively, while fortified estradiol was detected at 4 ng with iodine–starch and 10 ng with Corinth V. These levels of detection are below the tolerance levels for zeranol residues in bovine tissues (*i.e.* 150, 300 and 450 ppb for muscle, liver and kidney, respectively).

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