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Determination of estriol, estradiol, estrone, and progesterone in cosmetic products

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Synopsis

This report describes the development and validation of a reverse phase high-performance liquid chromatography (HPLC) method with UV detection for the determination of the hormones estriol, estradiol, estrone, and progesterone in topically applied products. The developed method was then used to conduct a postmarket survey of consumer products for these hormones. Each product was first mixed with Celite and then extracted with methanol. Extracts were cleaned on a Waters Oasis HLB solid phase extraction cartridge, and then analyzed using reversed phase HPLC. The analytes were separated using an Agilent Zorbax Eclipse XDB C8 (5 µm, 250 mm by 4.6 mm) analytical column and detected by their absorbance at 230 nm. Chromatographic separation was achieved by a 1.0-ml/min linear gradient from 30% acetonitrile and 70% water to 80% acetonitrile and 20% water over 30 min. A final 5 min hold time and a re-equilibration time of 10 min were used to prepare the column for subsequent analysis. Recovery from two different brand lotions spiked with three different levels of estriol, estradiol, estrone, and progesterone ranged from 81.8% to 101%. In this study, a total of 70 cosmetic products were surveyed. Twenty two (63%) of the 35 products were labeled as containing an estrogen and/or progesterone and also provided quantitative label information about the hormone ingredient. The most frequently labeled hormones were progesterone (66%), estriol (46%), estradiol (11%), and estrone (6%). Six products labeled as containing estriol were found to contain estradiol. An estrogen and/or progesterone were found in 34 products at concentrations ranging from 86.0 to 26,800 µg/g. Progesterone was not found in one product labeled as containing this hormone. An additional 35 products, which did not list hormones on their labels, were analyzed and estrogen or progesterone was not detected in these products.

INTRODUCTION

The three naturally occurring estrogens in humans are the steroids estriol, 17β-estradiol, and estrone, with estriol being the predominant estrogen during pregnancy, 17β-estradiol being the major estrogen in nonpregnant women of child-bearing age, and estrone being the primary estrogen in postmenopausal women (1,2) (Figure 1).

Another important class of hormones is progestagens (or progestogens), with progesterone being the naturally occurring progestagen in humans. The term "progestagen"

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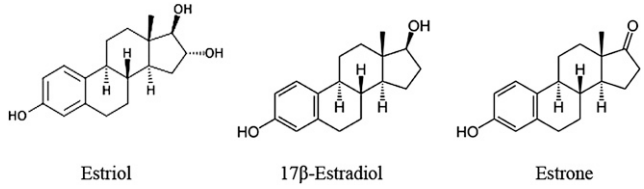


Figure 1. Naturally occurring estrogens.

derives from its function of maintaining pregnancy (progestational) and includes all steroids with a pregnane skeleton. However, synthetic progestagens are usually referred to as progestins (3) (Figure 2).

Many plants contain compounds with chemical structures similar to that of estrogens and progesterone. For example, the plant *Dioscorea mexicana*, a part of the yam family native to Mexico, contains a steroid, sapogenin called diosgenin, which can be converted in the laboratory to progesterone and other steroids. Some manufacturers have incorporated plant compounds, such as diosgenin, into their cosmetic products (Figure 3).

Studies of postmenopausal women show that low systemic estrogen levels are associated with dry skin, fine wrinkling, and declining levels of dermal collagen (4,5). Additional studies have indicated that estrogens and progesterone may have a beneficial effect on skin functions such as elasticity and water-holding ability and on dermal collagen levels (6–11). Beginning in 2002, large-scale epidemiological studies under the Women's Health Initiative have shown that hormone therapy poses risks as well as benefits. In particular, an increased risk of breast cancer and cardiovascular disease (12,13) as well as endometrial cancer, stroke, blood clots, ovarian cancer, and gallbladder disease have been associated with hormone therapies.

In addition, an explicit relationship has been proposed between excessive exposure to exogenous female sex hormones (including estriol, estradiol, estrone, and progesterone) and the risk of breast cancer in women (14,15). Because of the reported relationship between estrogen levels and skin attributes such as dryness and fine wrinkling (4) and the fact that creams sold for cosmetic uses are capable of producing significant drug exposures compared to oral drug products (16), there is a concern that topical application of products containing hormones could result in significant drug exposure. There are also reports in the literature of

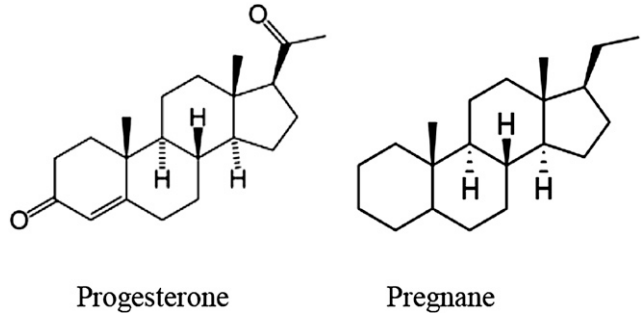
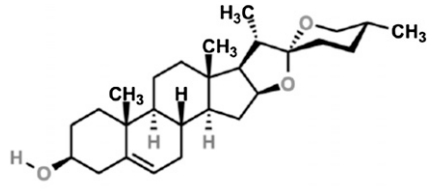


Figure 2. Progestagens.



Diosgenin

Figure 3. Steroid sapogenins.

cosmetic products containing estrogens (17,18) and other reports suggesting a possible linkage between these estrogen-containing cosmetic products and breast cancer (19).

In 2009, Olson *et al*. reported that women may be unintentionally exposing themselves to female sex hormones by using topical moisturizers and other topically applied products (17,18). They described analysis of a small number (n = 16) of topical moisturizers purchased or donated from department stores or chain drug stores. Each of the products was analyzed for estriol, estradiol, and estrone. Although none of the products declared any estrogen content on their list of ingredients, five of them were found to contain estriol and one contained estrone.

As noted earlier, studies have shown that estrogens and progesterone have a beneficial effect on skin functions such as elasticity and water-holding ability and on dermal collagen levels, and some cosmetic manufacturers may be marketing incompletely labeled or misbranded cosmetic products containing these hormones. In 1993, U.S. Food and Drug Administration (FDA) issued a final rule establishing that any topically applied over-the-counter drug product containing a hormone is not generally recognized as safe and is misbranded (20). Current FDA guidance on cosmetic labeling also states that products that are marketed as cosmetics but are also intended to treat or prevent disease, or affect the structure or functions of the human body, are also considered drugs and must comply with both the drug and cosmetic provisions of the law and provides, as an example, hormone creams, which are drugs as well as cosmetics (21).

Therefore, to accurately determine the extent and concentration of these hormones in typical cosmetic products used on the skin, a robust analytical method was developed to quantify estriol, estradiol, estrone, and progesterone in skin care products. This method was then used in a survey of a variety of skin care products (n = 70), including a few products containing wild yam extract. It has been reported that wild yam extract could potentially have an estrogenic effect, therefore there was interest in assessing these products (22) as well.

In addition to the present study, there are a limited number of reports in the scientific literature describing the analysis of cosmetic products and topically applied pharmaceutical preparations for female sex hormones (23–26). Havlikova *et al.* (23) used an Agilent Zorbax Stable Bond Cyano substituted analytical column (Agilent Technologies, Santa Clara, CA) with an isocratic mobile phase consisting of acetonitrile (27%), 0.085% phosphoric acid (63%), and tetrahydrofuran (10%) to chromatographically separate, without extraction or sample cleanup, estradiol and its degradation products in topical pharmaceutical gel preparations. Analytes were detected by their ultraviolet (UV) absorption at

225 nm. Feng *et al.* (24) used a monolithic capillary column and an 85% methanol and 15% 0.03 M phosphate solution to extract testosterone, methyltestosterone, and progesterone in liquid cosmetic products followed by isocratic high-performance liquid chromatography (HPLC) separation on a Hypersil ODS C18 (Thermo Electron Corporation, Waltham, Ma) column with 75% methanol and 25% water with UV detection at 245 nm.

De Orsi *et al.* (25) also used the Agilent Zorbax Stable Bond Cyano substituted analytical column to chromatographically separate minoxidil, progesterone, estrone, spironolactone, canrenone, hydrocortisone, and triamcinolone in liquid cosmetic creams and lotions, but used gradient elution starting at 90% water (with 0.1% trifluoroacetic acid) and 10% acetonitrile for 1 min, decreasing to 10% water and 90% acetonitrile in 40 min, and then returning to the initial condition in 10 min. Cosmetic samples were prepared for HPLC analysis by dissolution in methanol with sonication and centrifugation. UV detection was at 230, 254, and 280 nm. Novakova *et. al.* (26) determined concentrations of estradiol, its degradation product estrone, and the preservatives methylparaben and propylparaben in topical FDA-approved estrogen therapy gel preparations by extraction into a acetonitrile solution with sonication followed by centrifugation and then chromatographic separation on a Supelco Discovery C18 analytical column (Supelco, Bellefonte, PA) and an isocratic mixture of acetonitrile, methanol, and water in the ratio of 23:24:53 v/v with UV detection at 225 nm. Finally, there are many reports describing the analysis of other matrices for female sex hormones. Of these later reports, reports of the analysis of water samples for female sex hormones may be relevant (27–31).

Although the use of HPLC with UV detection for the analysis of cosmetic products and topically applied pharmaceutical preparations for various hormones, hormone degradation products, and preservatives is not novel, such reports are limited and where analysis of such products is reported, HPLC with UV detection has been the method of choice and the HPLC method presented in this manuscript, in terms of specific elution solvents, specific gradient program, and specific column used, is novel and original. In addition, the use of extraction from Celite with methanol followed by cleanup with a commercially available solid phase extraction cartridge and then chromatographic separation on an extra-dense boding (XDB) C8 analytical column, as applied to all four hormones estriol, estradiol, estrone, and progesterone in cosmetic product matrices, has not been reported previously to our knowledge. In comparison to that reported in references 23–26, the method presented in this report is applicable to a more diverse set of sample matrices and product types and a wider range of concentration levels. Moreover, in addition to the HPLC method presented, this report also provides important details regarding the label information content (e.g., hormones present and concentrations) and product use claims for surveyed cosmetic products.

EXPERIMENTAL

REAGENTS AND MATERIALS

The following reagents and materials were used: acetonitrile, methanol, water and Celite 545 [purchased from Fisher Scientific (Fairlawn, NJ)]. All solvents were of HPLC grade or better. Estriol (99%), estradiol (98%), estrone (99%), and progesterone (99%)

standards were purchased from Sigma Aldrich (Saint Louis, MO). The extraction tubes and filter disks were obtained from Supelco. Oasis HLB solid phase extraction cartridges were purchased from Waters Corporation (Milford, MA). A chromatographic separation was achieved using a Zorbax Eclipse XDB C8 (5 µm, 250 mm by 4.6 mm) analytical column obtained from Agilent Technologies.

ESTROGEN AND PROGESTERONE CALIBRATION STANDARDS

A stock solution of the three estrogens (~1.0 mg/ml each) was prepared by adding approximately 100 mg of each estrogen to a 100 ml volumetric flask and diluting to the mark with methanol. A separate stock solution containing progesterone (~1.0 mg/ml) was prepared by adding approximately 100 mg of progesterone to a 100 ml volumetric flask and diluting to the mark with methanol. Because of the wide range of possible concentrations in cosmetic products and because the linearity range for estriol, estradiol, and estrone (0.60 to 600 µg/g) is different from that for progesterone (0.30 to 300 µg/g), three different sets of working standards were prepared for estrogens and progesterone.

For the estrogens, one set was at approximately 0.60, 1.8, 3.6, and 6.0 µg/ml. A second set was at approximately 6.0, 18, 36, and 60 µg/ml and a third set was at 60, 180, 360, and 600 µg/ml. Similarly, for progesterone, one set was at approximately 0.30, 0.90, 1.8, and 3.0 µg/ml. A second set was at approximately 3.0, 9.0, 18, and 30 µg/ml and a third set was at 30, 90, 180, and 300 µg/ml.

Hormones were identified in sample extracts by comparing HPLC retention times with standards, and quantified using the standard calibration curve for each hormone. Sample extracts were diluted as necessary to assure that concentrations were in the linear range of the calibration curves.

SAMPLE PREPARATION

Approximately 300 mg of each sample was mixed thoroughly with about 1.5 g of Celite, and transferred to a 6-ml extraction tube containing a filter disk. The sample/Celite mixture was covered with a second filter disk and compacted firmly. The packed column was put on the top of a Waters Oasis HLB solid phase extraction cartridge for cleanup. The prepared extraction tube was eluted with sufficient methanol to obtain 10 ml of extract in a volumetric flask. The extracted sample was mixed prior to HPLC analysis. Sample extracts were diluted as necessary to assure concentrations were in the linear range of the calibration curves.

HPLC ANALYSIS

HPLC analyses were carried out on an Agilent 1100 HPLC instrument equipped with a an Agilent Zorbax Eclipse XDB C8 (5 µm , 250 mm by 4.6 mm) analytical column, a quaternary pumping system, a vacuum degasser, a UV photodiode array detector, and a computer with Agilent ChemStation (Agilent Technologies, Santa Clara, CA) software. Because each estrogen has a different absorbance profile, four different wavelengths, 230, 254, 280 and 300 nm, were evaluated for absorbance sensitivity and selectivity. For all four analytes, 230 nm was determined to be the best compromise.

Chromatographic separation was achieved by a linear gradient from 30% acetonitrile and 70% water to 80% acetonitrile and 20% water over 30 min. The final ratio was held for 5 min before returning to the starting conditions. A re-equilibration time of 10 min was used prior to the next injection. A constant flow rate (1.0 ml/min) and temperature (25°C) were used throughout the analysis. Hormone concentrations were determined in sample extracts and standards by duplicate injections of 20 µL.

The recovery of the estrogens and progesterone from two commercially available lotions which did not contain any estrogen or progesterone was determined by fortifying the lotions with 100, 1000 or 10,000 µg/g of each hormone followed by extraction and HPLC analysis as described above.

RESULTS AND DISCUSSION

The present study was initiated to develop a method for the extraction and HPLC separation of the hormones estradiol, estriol, estrone, and progesterone. Each analyte peak was baseline separated and could be unambiguously quantified. Each hormone was identified by comparison of peak retention times and UV spectra with known standards. Chromatogram A in Figure 4 shows the chromatographic separation and elution order of estriol, estradiol, estrone, and progesterone as standard solutions in methanol, with retention times of 5.4, 12.9, 15.7, and 21.8 min, respectively. As expected, the retention time was found to be inversely correlated with the number of alcohol groups present in the analyte as an indicator of the analyte's polarity. All four hormone analytes were stable with no evidence of degradation, either during extraction or chromatographic separation.

Chromatograms B through E show typical chromatographic separations of extracts of cosmetic products containing estriol, estradiol, estrone, and/or progesterone. As shown by the five chromatograms in Figure 4, retention times observed for each hormone were very consistent and did not vary with sample matrix.

The linearity ranges for estriol, estradiol, and estrone were from 0.60 to 600 µg/g, while the linearity range for progesterone was from 0.30 to 300 µg/g. The limit of quantification (10 times baseline noise) ranged from 0.46 to 1.0 µg/g. The limit of detection (3.3 times baseline noise) ranged from 0.15 to 0.30 µg/g. Regression correlation coefficients were better than 0.995.

Recovery experiments were performed on two commercially available lotions (not among the products analyzed in the study) which did not contain estriol, estradiol, estrone, or progesterone. Table I shows specific recoveries for these sample matrices spiked with three different concentrations (100, 1000, 10000 µg/g) of estriol, estradiol, estrone, and progesterone. Recoveries of the four hormones ranged from 81.8% to 101%. A single liquid/solid extraction of 10 ml was chosen for simplicity and rapidity since increasing the volume of methanol extractant or repeat extractions was found not to significantly increase the observed recovery. For lotion A, a second recovery experiment was completed on a different day (Table I).

A total of 70 products marketed as cosmetics were selected and purchased from the Internet. The 70 products, as shown in Table II, were creams, lotions, moisturizers, oils, and extracts and had labels claiming a diverse range of product types, hormones present, and uses. Among the 35 products labeled as containing estrogen and/or progesterone, 22 (63%)

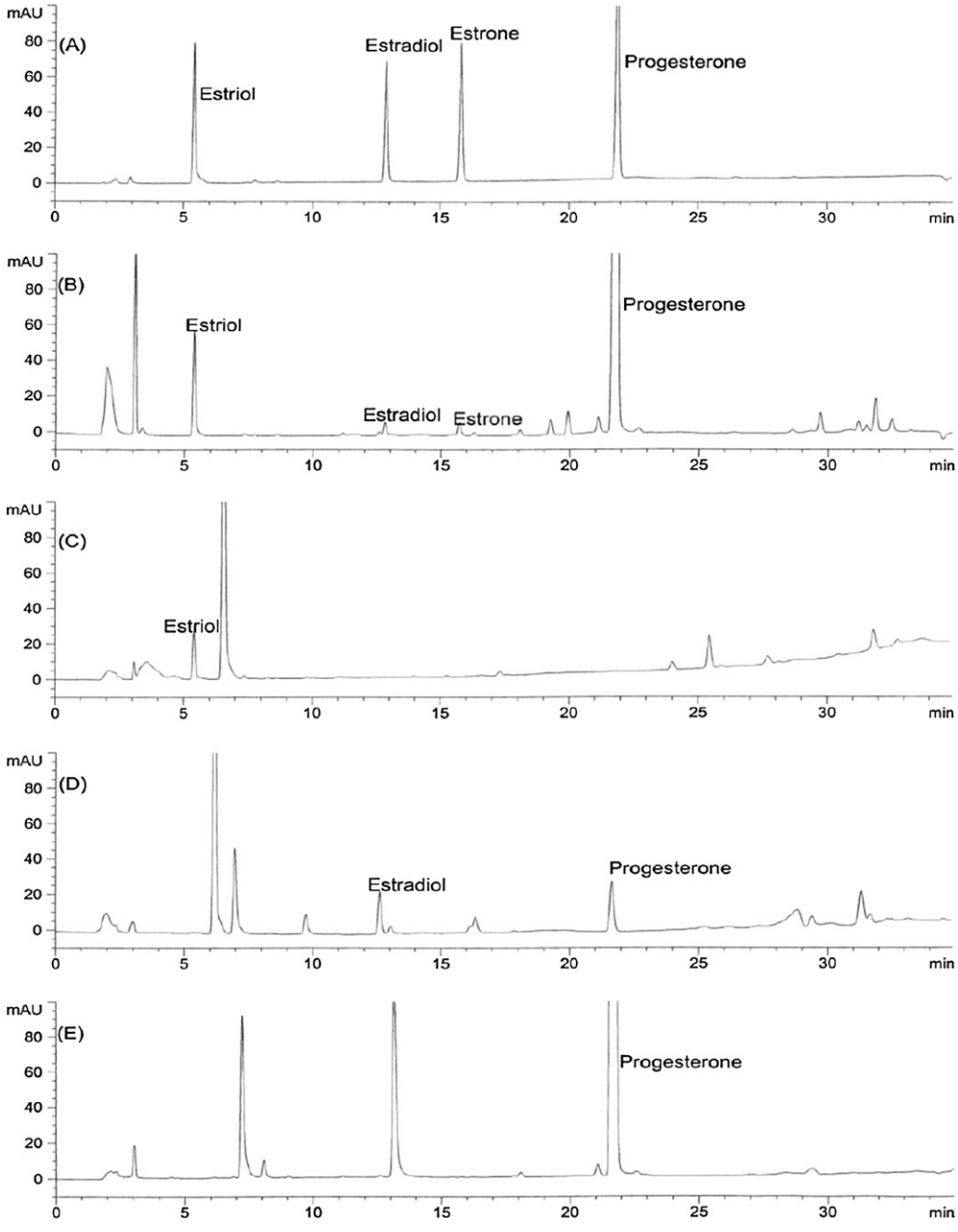


Figure 4. HPLC separation of a standard solution containing estriol, estradiol, estrone and progesterone in methanol (A). Typical chromatograms of sample extracts (B), (C), (D) and (E).

of the labels provided quantitative information. The labeled hormones were progesterone (23 out of 35 or 66%), estriol (16 out of 35 or 46%), estradiol (4 out of 35 or 11%), and estrone (2 out of 35 or 6%). For these 35 products, indicated uses fell into four broad categories: (a) relief for or treatment of premenstrual syndrome (PMS), menstruation, perimenopause, menopause, postmenopause; (b) hormone or "women's" balance; (c)

Table I
Mass Recovery of Estriol, Estradiol, Estrone, and Progesterone from Two Lotions[a]

		Percent recovery for each hormone analyte			
Product	Spike (μg/g)	Estriol (%)	Estradiol (%)	Estrone (%)	Progesterone (%)
Lotion A	100	97.2	101	97.4	92.5
	1000	92.3	94.5	92.4	104
	10000	96.2	95.7	101	97.2
Lotion B	100	90.0	101	98.6	94.0
	1000	87.8	81.8	86.9	87.4
	10000	97.7	94.8	101	96.7

[a]Lotions A and B are different products. Values for lotion A are averages of two separate extractions with duplicate injections of each extract. Values for lotion B are averages for duplicate injections of a single extraction.

antiaging, and (d) for improved skin condition (e.g., radiant complexion, luminous, and supple).

Among the 35 products that did not list hormones on their labels were five products whose labels indicated the presence of plant compounds (e.g., phytoestrogens) extracted from wild yams (e.g., *Dioscorea villosa*). These products also indicated use for menopause and for antiaging and antiwrinkle. For the remaining 30 products that did not list hormones on their labels, indicated uses included antiaging, antiwrinkle, line repair, and improved skin condition (e.g., skin brightness and elasticity, youth activating, moisturizing, lubrication, hydration).

Among all 70 products, 43 products included product use warnings or instructions on the label. Most, though not all, included general recommendations such as "for external use only," "avoid contact with the eyes," and "discontinue use if you experience irritation or discomfort." Many also included specific recommendations not to use during pregnancy or lactation or to keep out of reach of children. Three noted the presence of a chemical known by the State of California to cause cancer (i.e., progesterone). One recommended consultation with a health care professional before use and another stated that it was "for licensed physicians only."

As shown in Table III, estrogens and/or progesterone were found in 34 out of 35 products (whose labels indicated the presence of a specific hormone) at concentrations ranging from 86 to 26,800 μg/g. Six products labeled as containing estriol were found to contain estradiol. Progesterone was not found in one product labeled as containing this hormone. The 35 products that did not list hormones on their labels were found not to contain estrogen or progesterone.

CONCLUSION

An analytical method for the determination of estriol, estradiol, estrone, and progesterone in cosmetic products has been developed, validated, and used to conduct a limited survey of products marketed as cosmetics for estrogens and progesterone. In this survey, no products were found to contain hormones that were not listed on the product label.

Table II
Sample Descriptions

Product ID	Product type	Label information on hormones	Use	Use direction	Product use warning
1	Transdermal cream	Estriol Estradiol Estrone Progesterone	Control of menopausal symptoms	Yes	Yes
2	Face and body cream	Progesterone	PMS and menopause skin care	Yes	Yes
3	Natural progesterone cream	Progesterone	Menopause/perimenopause support	Yes	Yes
4	Progesterone cream	Progesterone	Menopause/perimenopause support	Yes	Yes
5	Nature estriol cream	Natural estriol	Control of menapausal symptoms	Yes	Yes
6	Estrogen body cream	Natural estriol	Control of menapausal symptoms	Yes	Yes
7	Natural progesterone body cream	Natural progesterone from soy, wild yam root extract)	For premenapause/menopause/post menopause women	Yes	Yes
8	Natural progesterone body cream	Natural progesterone (derived from Mexican wild yam root)	For menstruating women	Yea	Yes
9	Estrogen body cream	Natural estriol	Control of menopausal symptoms	Yes	Yes
10	Natural progesterone body cream	Natural progesterone	For premenopause/menopause/post menopause women	Yes	Yes
11	Rejuvenate antiaging estriol face cream	Estriol (natural estrogens)	Antiaging cream may reduce wrinkle depth and pore size	Yes	Yes
12	Skin care cream	Estriol	Advanced contouring peptides and cellular rejuvenators for luminous and supple skin	Yes	Yes
13	Progesterone enhanced skin therapy cream	Progesterone	Skin therapy		
14	Estro pro cream	Estriol Estradiol Estrone Progesterone		Yes	
15	Age intervention face cream	Estriol Progesterone	Age intervention face cream	Yes	
16	Skin enhancing beauty cream	Estriol Progesterone	Skin enhancing beauty cream to tone skin and stimulate the skin with a minimal effect on hormone balance	Yes	Yes

Table II
Continued

Product ID	Product type	Label information on hormones	Use	Use direction	Product use warning
17	Natural progesterone balancing cream	450 mg USP (United States Pharmacopeia) progesterone per ounce	Women's balancing cream	Yes	Yes
18	Naturals estriol cream	150 mg USP estriol (0.25%)	Women's balancing cream	Yes	Yes
19	Natural progesterone liposomal skin cream	Natural progesterone (from wild yam)	Women seeking natural hormone	Yes	Yes
20	Essential moisture plus cream	Estriol progesterone	Alternative moisture cream for women	Yes	Yes
21	Rejuvenate antiaging estriol face cream	0.3% Estriol	Antiaging face cream	Yes	
22	Naturally radiant estrogen crème	Estriol USP	Women's balancing cream	Yes	Yes
23	Natural progesterone balance crème	Progesterone USP 1.85% (1,020 mg—wild yam derived)	For menstruating women	Yes	Yes
24	BiEstro-Care body cream	Estriol (USP), 24 mg/ounce Estradiol (USP), 6 mg/ounce	Women's balancing cream	Yes	Yes
25	ProgestoCell natural cream formula	Progesterone USP	Women's balancing cream	Yes	Yes
26	Pegesterone enhanced skin therapy cream	500 mg Progesterone USP per ounce	Pegesterone natural liposome cream with DLPA	Yes	Yes
27	Natural estriol cream	Estrio	Women's balancing cream	Yes	Yes
28	Natural progesterone cream with phytoestrogens	Progesterone (micronized) USP	For menstruating, menopausal or non-menstruating women	Yes	Yes
29	Progesta-care with natural progesterone body cream	Progesterone USP (480 mg/ounce)	Women's balancing cream	Yes	Yes
30	Essential moisturizing cream	Progesterone	Women's balancing cream	Yes	
31	Natural progesterone body creme	Progesterone	Balancing cream	Yes	Yes
32	Real estrogen oil	Micronized natural estriol USP	Women's balancing oil	Yes	Yes

Table II
Continued

Product ID	Product type	Label information on hormones	Use	Use direction	Product use warning
33	Natural progestelle skin oil	800 mg Of pharmacetical grade USP Natural progesterone (from yams) per ounce of oil. coconut oil	For menses/menopause/ postmenopause women	Yes	Yes
34	Estriol-care body cream	Estriol	Estriol-care body cream (with natural Estriol)	Yes	Yes
35	Phytoestrogen body cream	Plant compounds structurally similar to estrogen	Menopause/perimenopause support	Yes	Yes
36	Rejuvenation cream	Natural progesterone (yam derived)	For a radiant complexion	Yes	
37	Age-diffusing serum	Phytoestrogens (*D. villosa*, wild yam root extract)	Combats effects of hormonal aging	Yes	Yes
38	Multi wild yam complex	*D. villosa*, wild yam root extract		Yes	
39	Youth activating cream serum	None	Youth activating cream serum	Yes	
40	Celluar radiance eye cream	Wild yam root extract, but does not specify any hormones	Time-corrective and restorative treatment for eyes	Yes	Yes
41	Skin care cream		Replenish, repair and rejuvenate skin	Yes	Yes
42	Antiaging moisturizer cream	None	Antiaging moisturizer	Yes	Yes
43	Antiwrinkle day cream	None	Ultra correction line repair for face and neck		
44	Day cream	None	Antiwrinkle and firming moisturizer	Yes	Yes
45	Cellular radiance cream	None	Skin creme for face	Yes	Yes
46	Skin repair emollient	None	Activate skin repair cells for healthy younger skin	Yes	
47	Antiaging moisture cream	None	Long-lasting moisture retention	Yes	Yes
48	Firm and repair treatment serum	None	Antiaging complex	Yes	Yes
49	Day and night revitalizing moisturizer	None	Lubricates skin with natural emollients		Yes

Table II
Continued

Product ID	Product type	Label information on hormones	Use	Use direction	Product use warning
50	Youth enhancing lotion with antioxidant	None		No	Yes
51	Skin care cream	None	Advanced antiaging moisturizer	Yes	
52	Antiaging Serum	None	Antiaging serum	Yes	
53	Youth activating concentrate	None	For younger skin	Yes	
54	MSM (Methyl-Sulfonyl-Methane), vitamin E body lotion	None	Natural radiance apply cream		
55	Mega Rich intensive antiaging cellular creme	None	Antiwrinkle/antiaging	Yes	
56	Pomegranate antiwrinkle cream	None	Makes rough and tired skin smooth and glossy	Yes	Yes
57	Advanced biogen selection cream	None	For tired skin in need of regeneration apply cream		
58	Rapid wrinkle repair night moisturizer	None	Antiwrinkle	Yes	Yes
59	Face lightening cream	None	Apply cream		
60	Moisturizing lotion	None	Moisturizer	Yes	
61	Pomegranate sunflower lotion	None	Moisturizer	Yes	Yes
62	Perfecting day cream broad-spectrum SPF (Sun Protection Factor) 30/PA+++	None	Moisturizer/prevents sun burn	Yes	Yes
63	Antiaging night firming treatment cream	None	Antiaging	Yes	
64	Night cream	None	Antiaging cream	Yes	Yes
65	Neck cream	None	Antiaging for the neck	Yes	Yes

Table II
Continued

Product ID	Product type	Label information on hormones	Use	Use direction	Product use warning
66	Perfuming body cream	None	Perfumed body cream application, cream enhances diffusion on the skin		
67	Age-diffusing serum	*D. villosa* (wild yam) root extract but no specific hormones	Antiwrinkle/antiaging	Yes	Yes
68	Dry skin cream	None	Deep hydration for smooth, soft, radiant skin (moisturizer apply cream)	Yes	
69	Refining moisture cream complex	None	Refining moisture cream complex		
70	Skin moisturizer lotion	None	CK one skin moisturizer lotion		

Table III
Hormone Concentrations Found in Products Surveyed (μg/g)[a]

Product ID	Estriol (μg/g)	Estradiol (μg/g)	Estrone (μg/g)	Progesterone (μg/g)
1	747	86.0	99.0	18,200
2	ND[b]	ND	ND	14,600
3	ND	ND	ND	11,030
4	ND	ND	ND	12,700
5	ND	1120	ND	ND
6	ND	1030	ND	ND
7	ND	ND	ND	16,200
8	ND	ND	ND	14,800
9	ND	863	ND	ND
10	ND	ND	ND	16,400
11	ND	429	ND	257
12	388	ND	ND	ND
13	ND	ND	ND	14,500
14	709	110	138	17,800
15	ND	1300	ND	1050
16	ND	172	ND	264
17	ND	ND	ND	14,200
18	2620	ND	ND	ND
19	ND	ND	ND	18,600
20	651	ND	ND	5030
21	ND	1280	ND	ND
22	905	ND	ND	ND
23	ND	ND	ND	17,050
24	796	282	ND	ND
25	ND	ND	ND	19,200
26	ND	ND	ND	17,800
27	564	ND	ND	ND
28	ND	ND	ND	14,400
29	ND	ND	ND	15,500
30	ND	ND	ND	23,300
31	ND	ND	ND	14,500
32	696	ND	ND	ND
33	ND	ND	ND	26,800
34	554	ND	ND	ND
35–70	ND	ND	ND	ND

[a]Average of two independent samples taken of each product, extracted, and then analyzed with two injections per extract.
[b]Below the limit of quantification.

This survey provides current data on the use of estrogenic hormones in moisturizers, antiaging products, and other product types marketed as cosmetics and will be used to evaluate human exposure to hormones from these products. The FDA does not allow the

use of therapeutically significant levels (3%) of estrogen and related substances in cosmetic products. The agency is continuing to evaluate new data on this complicated issue to monitor the safety of these products, but to date, has found no convincing evidence that the ingredients used in such products pose a health risk to consumers.

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