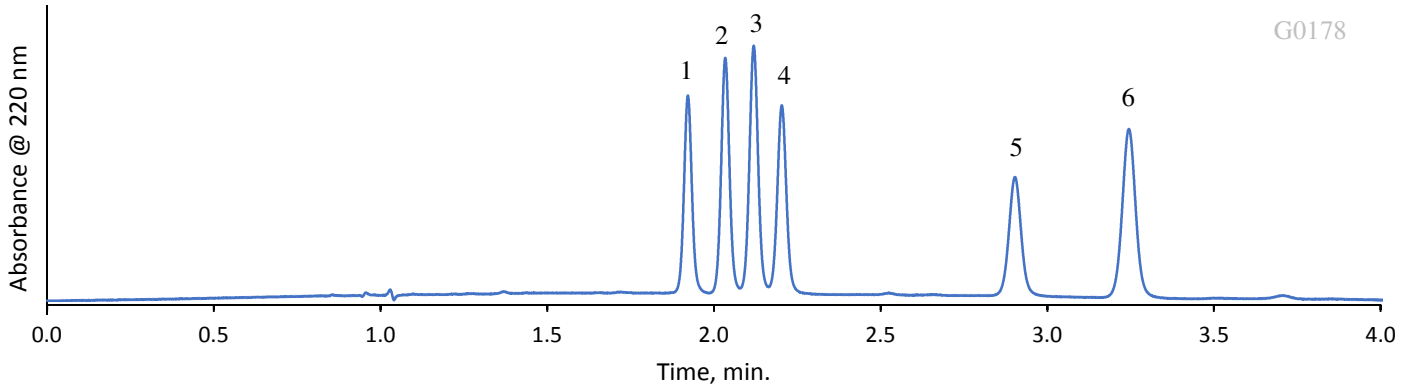
HALO® | Fused-Core® Particle Technology

Application Note: 184-STR

Separation of Glucocorticoids on HALO® C30



TEST CONDITIONS:

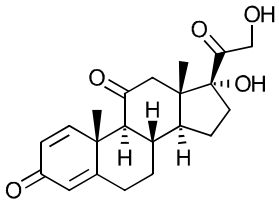
Column: HALO 160Å C30, 2.7 µm, 4.6 x 150mm
Part Number: 92114-730
Mobile Phase A: Water
Mobile Phase B: 50/50 Acetonitrile/ Methanol
Isocratic: 50% B
Flow Rate: 1.5 mL/min
Pressure: 355 bar
Temperature: 50°C
Detection: UV 220 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: Acetonitrile
Data Rate: 40 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

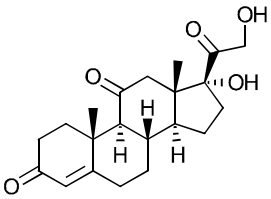
Glucocorticoids are a class of steroid drugs that have anti-inflammatory and anti-allergy benefits, as well as anti-lymphatic cancer uses. This mixture of six glucocorticoids is separated with high resolution in less than four minutes on a HALO C30 column.

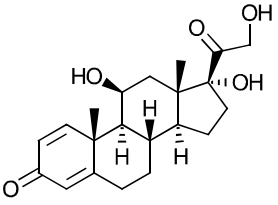
PEAK IDENTITIES:

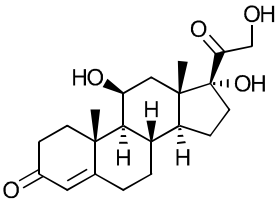
1. Prednisone
2. Cortisone
3. Prednisolone
4. Hydrocortisone
5. Dexamethasone
6. Corticosterone

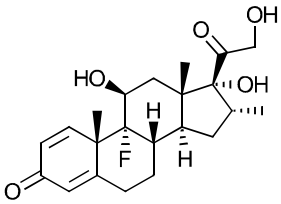
STRUCTURES:

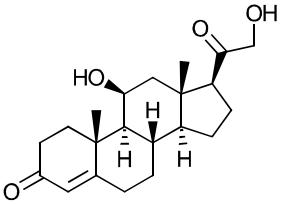

Prednisone


Cortisone


Prednisolone


Hydrocortisone


Dexamethasone


Corticosterone

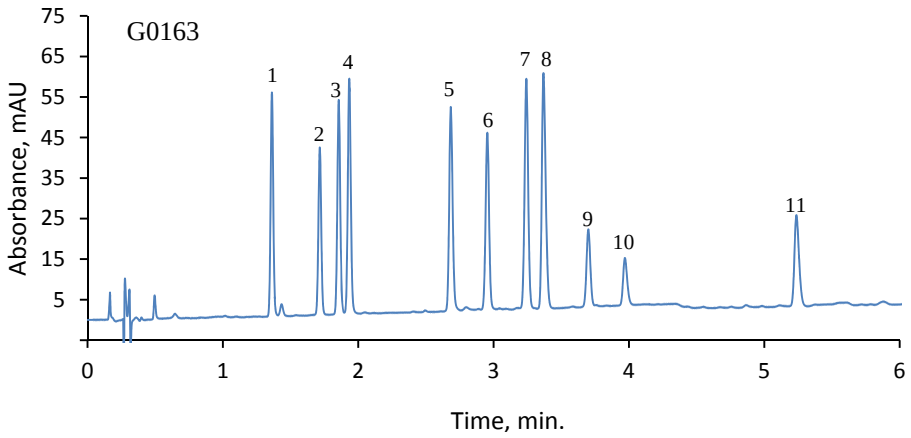
advancedmaterialstechnology
www.advanced-materials-tech.com

FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

HALO | Fused-Core® Particle Technology

Application Note: 169-STR

Separation of Steroids on HALO 90Å Biphenyl



PEAK IDENTITIES:

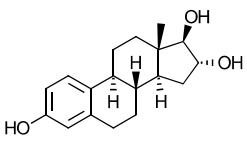
1. Estriol
2. Hydrocortisone
3. Prednisone
4. Cortisone
5. Corticosterone
6. β-Estradiol
7. Cortisone Acetate
8. Testosterone
9. 17-α-Hydroxyprogesterone
10. 11-Deoxycorticosterone
11. Progesterone

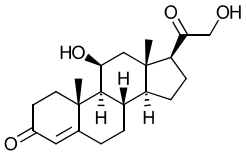
TEST CONDITIONS:

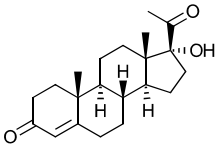
Column: HALO 90Å Biphenyl, 2.7 µm, 4.6 x 50mm
Part Number: 92814-411
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Gradient: 20-60% B in 6 minutes
Flow Rate: 1.85 mL/min
Initial Pressure: 344 bar
Temperature: 30°C
Detection: UV 215 nm, PDA
Injection Volume: 4 µL
Sample Solvent: Acetonitrile:water, 37.5:62.5
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

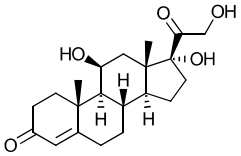
A mixture of 11 steroids is separated using a 6-minute gradient on a HALO 90Å Biphenyl column. The chromatogram shows very good resolution between all peak pairs with excellent peak shape and high efficiency.

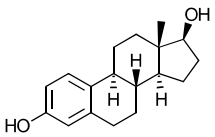
STRUCTURES:

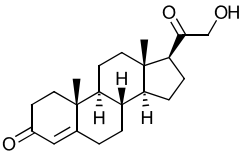

Estriol

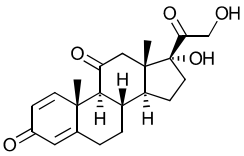

Corticosterone

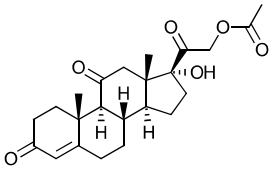

17α-Hydroxyprogesterone

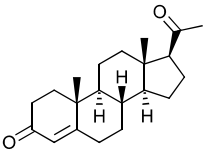

Hydrocortisone

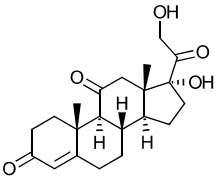

ß-Estradiol

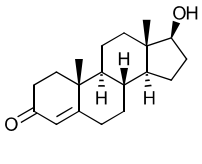

11-Deoxycorticosterone


Prednisone


Cortisone Acetate


Progesterone


Cortisone


Testosterone

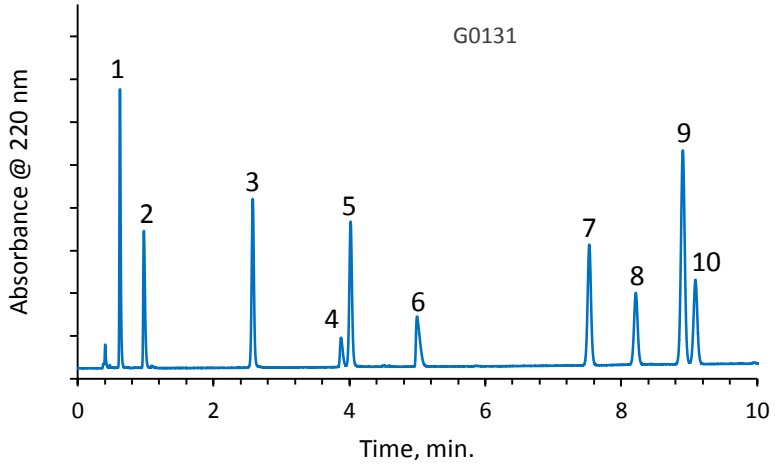
advancedmaterialstechnology
www.advanced-materials-tech.com

FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

HALO | Fused-Core® Particle Technology

Application Note: 142-STR

Separation of Steroid Hormones and Hormone Conjugates on HALO C18



PEAK IDENTITIES:

1. Estriol-3-(β-D-glucuronide)
2. Estriol-3-Sulfate
3. Estrone-3-(β-D-glucuronide)
4. β-Estradiol-3-Sulfate
5. Estriol
6. Estrone-3-Sulfate
7. β-Estradiol
8. α-Estradiol
9. Androstenedione
10. Estrone

TEST CONDITIONS:

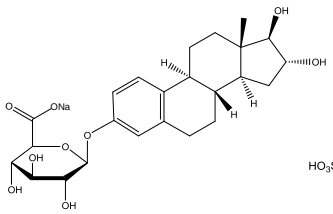
Column: HALO C18, 2.1 x 100 mm, 2.7 µm
Part Number: 92812-602
A= 10 mM phosphate buffer, (pH=7)
B= acetonitrile
Gradient:

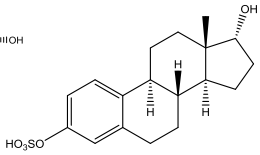
Time (min.)	%B
0.0	20
10.0	43

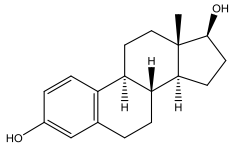
Flow Rate: 0.5 mL/min.
Pressure: 366 bar
Temperature: 25 °C
Injection Volume: 4 µL
Sample Solvent: 84/16 water/acetonitrile
Detection: UV 220 nm, PDA
Data Rate: 40 Hz
Response Time: 0.05 sec.
Flow Cell: 1 µL
LC System: Nexera X2

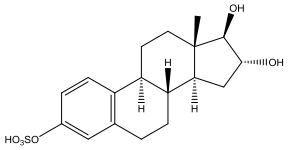
Steroid hormones and hormone conjugates are monitored for a variety of medical reasons. This fast separation of ten estrogens and estrogen-related compounds was accomplished with a HALO C18 column.

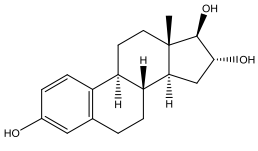
STRUCTURES:

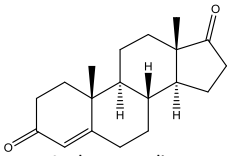

Estriol-3-(β-D-glucuronide)

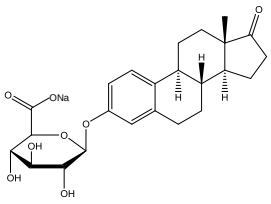

β-Estradiol-3-Sulfate

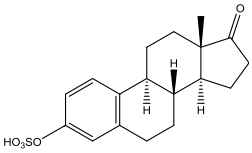

α-Estradiol

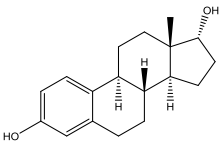

Estriol-3-Sulfate

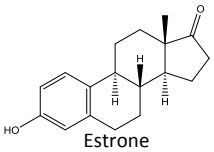

Estriol


Androstenedione


Estrone-3-(β-D-glucuronide)


Estrone-3-Sulfate


β-Estradiol

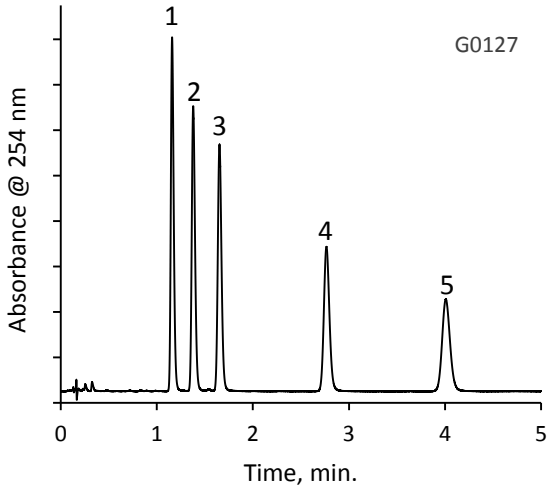

Estrone

advancedmaterialstechnology
www.advanced-materials-tech.com

FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

Application Note: 139-STR

Separation of Anabolic Steroids on HALO 2 C18



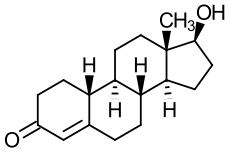
PEAK IDENTITIES:

1. Nandrolone
2. Methandienone
3. Testosterone
4. Epitestosterone
5. Norethandrolone

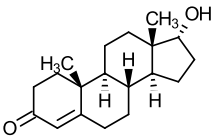
TEST CONDITIONS:

Column: HALO 2 C18, 2.1 x 50 mm, 2 µm
Part Number: 91812-402
Mobile Phase: 70/30 water/acetonitrile
Flow Rate: 0.8 mL/min.
Pressure: 476 bar
Temperature: 40°C
Injection Volume: 2 µL
Sample Solvent: 37.5/62.5 water/organic solvent (acetonitrile, methanol, and 1,2-dimethoxyethane)
Detection: UV 254 nm, PDA
Data Rate: 80 Hz
Response Time: 0.02 sec.
Flow Cell: 2 µL micro cell
LC System: Agilent 1200 SL

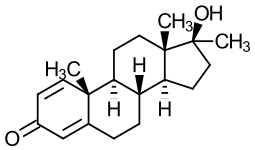
STRUCTURES:



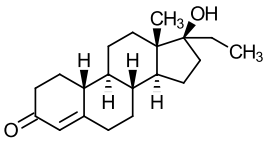
Nandrolone



Epitestosterone

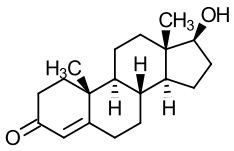


Methandienone



Norethandrolone

Screening for steroid use is common in both sports and medicine. These five anabolic steroids are separated in less than 5 minutes using a 2-micron HALO 2 C18 column.



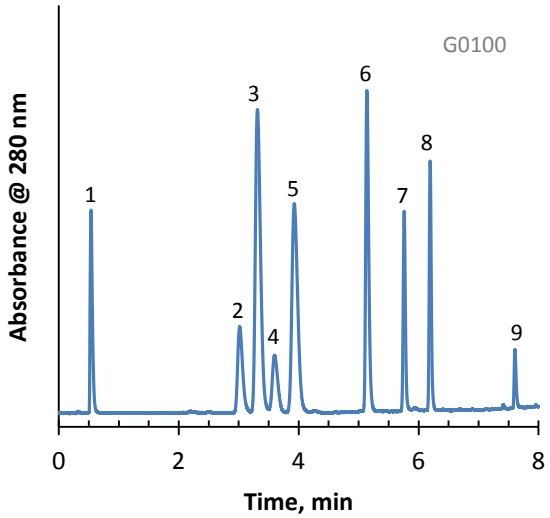
Testosterone

advancedmaterialstechnology
www.advanced-materials-tech.com

FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

Application Note: 116-STR

Separation of Steroids on HALO 2 PFP



PEAK IDENTITIES:

1. Uracil
2. Hydrocortisone
3. Prednisolone
4. Cortisone
5. Prednisone
6. Dexamethasone
7. β-Estradiol
8. Estrone
9. Halcinonide

TEST CONDITIONS:

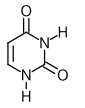
Column: 3.0 x 50 mm, HALO 2 PFP
Part Number: 91813-409
Mobile Phase A: water
Mobile Phase B: methanol
Gradient:

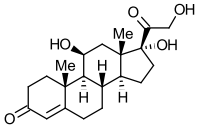
Time (min.)	%B
0	47
3	47
8	88

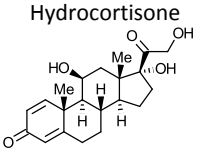
Flow Rate: 0.4 mL/min.
Pressure: 180 bar initial
Temperature: 35°C
Detection: UV 280 nm, VWD
Injection Volume: 2 µL
Sample Solvent: methanol
Response Time: 0.02 sec.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
ECV: ~14 µL

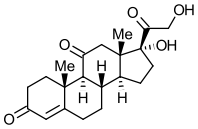
HALO 2 PFP is useful in the separation of closely related steroids. Even though this separation was run on a system with 14 µL of extra-column volume, there is sufficient efficiency with a HALO 2 column to separate the first four steroids during the isocratic hold at the beginning of the run.

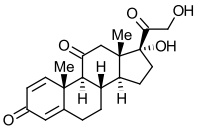
STRUCTURES:

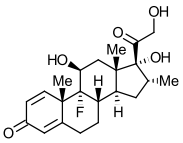

Uracil

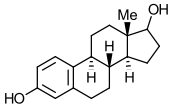

Hydrocortisone

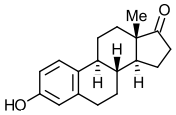

Prednisolone

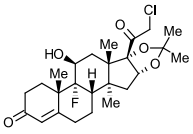

Cortisone


Prednisone


Dexamethasone


β-Estradiol


Estrone

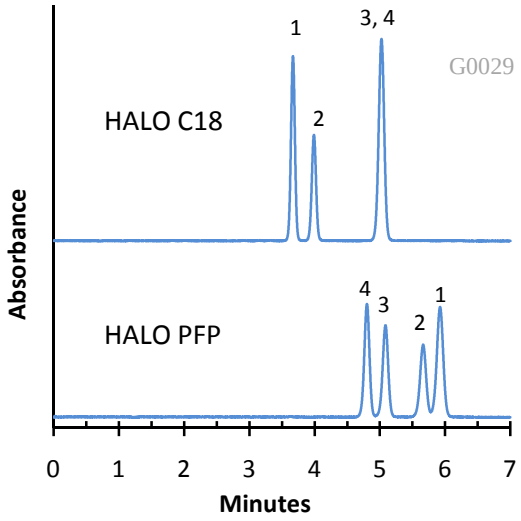

Halcinonide

advancedmaterialstechnology
www.advanced-materials-tech.com

FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

Application Note: 047-STR

Separation of Structurally Similar Steroids on HALO C18 and PFP



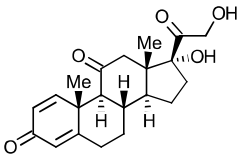
PEAK IDENTITIES:

1. Prednisone
2. Cortisone
3. Prednisolone
4. Hydrocortisone

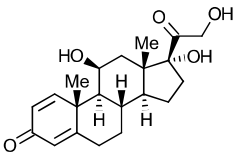
TEST CONDITIONS:

Columns: 4.6 x 100 mm, HALO C18
4.6 x 100 mm, HALO PFP
Part Numbers: C18, 92814-602
PFP, 92814-609
Mobile Phase: 50/50: water/methanol
Flow Rate: 1.0 mL/min.
Pressure: About 230 Bar
Temperature: 35°C
Detection: UV 240 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 80% methanol in water
Response Time: 0.02 sec.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
ECV: ~14 µL

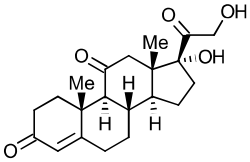
STRUCTURES:



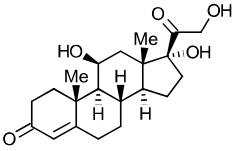
Prednisone



Prednisolone



Cortisone



Hydrocortisone

The unique selectivity of HALO PFP is useful in the separation of the closely related steroids prednisolone and hydrocortisone. The electron-deficient ring structure of the perfluorophenyl group aids in separating compounds through pi-pi interactions with the sample.

advancedmaterialstechnology
www.advanced-materials-tech.com

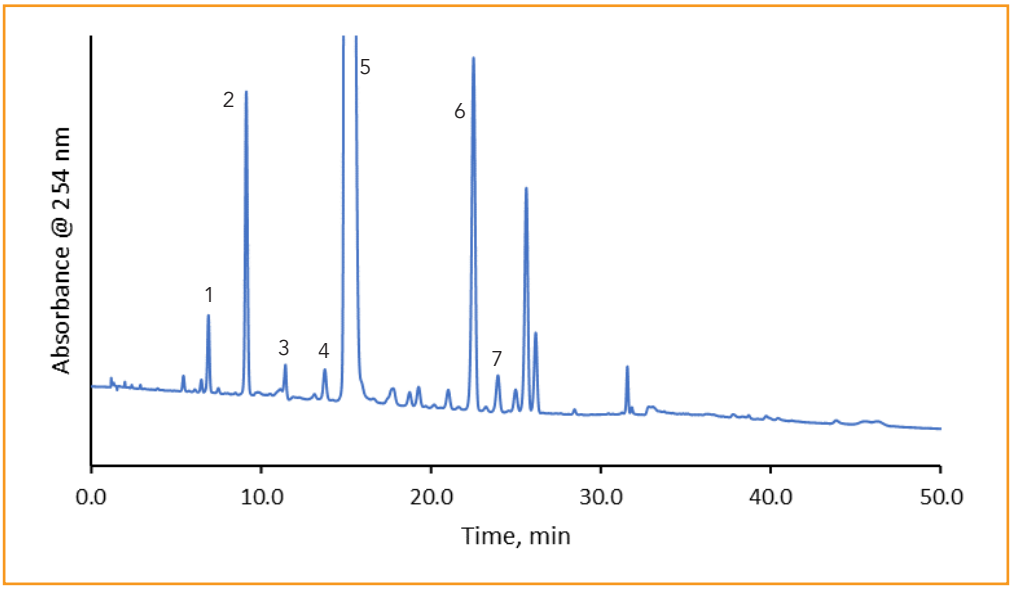
FOR MORE INFORMATION OR TO PLACE AN ORDER, CONTACT:

HALO®

PHARMACEUTICALS

Dexamethasone Sodium Phosphate (EP 10.0)

257-S



TEST CONDITIONS:

Column: HALO 90 Å C8, 5 µm, 4.6x150 mm
Part Number: 95814-708
Mobile Phase A: 300 mL solution A, 350 mL water, 350 mL MeOH, pH: 3.8
Mobile Phase B: 300 mL solution A, pH: 4, 700 mL MeOH solution A: dissolve 7.0 g of ammonium acetate in 1000 mL water

Gradient:

Time	%B
0.0	10
3.5	10
23.5	40
34.5	95
50.0	95

Flow Rate: 1.0 mL
Pressure: 209 bar
Temperature: 30 °C
Detection: UV 254 nm, PDA
Injection Volume: 20 µL Reference Solution B
Sample Solvent: mobile phase A
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

PEAK IDENTITIES

1. Impurity C
2. Impurity D
3. Impurity E
4. Impurity F
5. Dexamethasone sodium phosphate
6. Impurity A
7. Impurity G

Dexamethasone relieves inflammation and is used to treat several conditions such as arthritis, allergic reactions, and bowel disorders. A HALO 90 Å C8, 5µm column is used to separate dexamethasone and its impurities following the European Pharmacopoeia 10.0 method.

advancedmaterialstechnology
Made in the USA | fused-core.com

INNOVATION YOU CAN TRUST,
PERFORMANCE YOU CAN RELY ON

HALO®

TECHNICAL REPORT: AMT-TRFB062101

TITLE: **HPLC ANALYSIS OF STEROIDS IN ORGANIC VS. NON-ORGANIC BEEF**

MARKET SEGMENT: FOOD/BEVERAGE

AUTHOR:
Andrew Harron Ph.D., Application Scientist

ABSTRACT

Organic and non-organic ground beef samples were extracted and analyzed for steroids using the HALO 90 Å C18 column. A panel of steroids, which consisted of a mixture of growth promotors and those used for therapeutic purposes, was screened for in two different samples of ground beef, an organic (OGB) and a non-organic brand (NOGB). The steroid panel consisted of Estradiol 17ß, testosterone, progesterone, zeranol, melengestrol acetate (MGA), Aldosterone, corticosterone, and 17 A-methyltestosterone. The steroids were separated, detected and quantitated with only progesterone detected in both beef samples, with levels of 60 ng/mL for the OGB and 381ng/mL for the NOGB. The HALO 90 Å C18 column proved to be an ideal solution for the separation of steroids in beef samples.

INTRODUCTION

Steroid hormones are lipophilic, naturally occurring, small molecule compounds, which regulate many essential functions in the human body. Increased exposure to high levels of steroids, however, has been linked to cardiovascular system damage and cancer.[1] This is an area of concern, as synthetic hormones and steroids have found application in the food industry, and many animals that are in the food chain have been exposed to high levels of steroids during their lifetime.[1]

For over fifty years, the Food and Drug Administration (FDA) has approved the use of a number of steroids in beef cattle, including natural estrogen, progesterone, testosterone, and their synthetic versions such as trenbolone acetate (TBA).[1-5] The function of these drugs is to increase growth rate and the efficiency by which the animals convert the feed they eat into muscle/meat. The drugs are usually administered as implants (dosing of 100-200 days), which are placed under the skin on the back side of the animal's ear. The implants dissolve slowly under the skin and are not removed.[2-3] Although cooking the meat does have some effect on the stability of the steroids in beef, it does not eliminate the exposure, as many steroids are stable at elevated temperatures.[6]

The acceptable levels of steroids in beef are a mixture of complex and highly contested political issues, particularly by the United States and Canada, which are two of the highest beef exporting countries in the world.[7-10] The FDA regulation for acceptable levels of steroids in beef is ambiguous, and focuses mainly on trace residues affecting human life after consumption of the beef.[2,7,10] The EU has adopted more stringent guidelines, and in 1981, prohibited the use of growth producing hormones in their beef supply.[7-10] Examples for these kinds of growth promoters are estradiol 17ß, testosterone, progesterone, zeranol, TBA and melengestrol acetate (MGA). This resulted in millions of pounds of beef from the United States and Canada banned from the EU marketplace.[3,4,7-10] This was challenged by the United States and Canada and ultimately reversed. However, the EU later mandated a

KEY WORDS:

Steroid implant, cattle, LCMS, Steroids in beef, cancer, HALO 90 Å C18 column, lipophilic small molecules

new assessment of the risks to human health from hormone residues in bovine meat, and adopted directive 2003/74/EC, which specifically targets both domestic and imported meat and meat products treated with hormones used for growth promotion. [4,7-10] This allows the EU to prohibit imported food which is deemed unsafe for public consumption, however it focuses mainly on estradiol 17ß, TBA, and MGA.[9] Progesterone and testosterone, as naturally occurring hormones, are often times not considered as toxic as their synthetic counterparts, however in the case of progesterone, high levels of exposure can present many health risks.[11-16]

Progesterone is a naturally occurring hormone, but can also be administered as part of a growth hormone treatment implant.[1-3] Progesterone has been linked to a variety of diseases, including breast cancer, particularly in postmenopausal women, problems with metabolism, the central nervous system, and the respiratory system.[11-16] Progesterone is not carcinogenic, however does affect endocrine activity with steroid hormone receptor interactions, and can increase tumor activity in endocrine tissues, particularly in ovarian tissue.[11,17] The DHHS/National Toxicology Program concluded that under increased levels of exposure, steroidal hormones, including progesterone, do display carcinogenic activity.[17] Therefore, increased levels of exposure to progesterone, and sex steroids in general, is of rising concern.[11]

Reliable beef analysis is critical for the detection and quantification of steroids in beef, as increased exposure to steroids has triggered many negative health effects.[11-17] Here we present the HALO 90 Å C18 column for the analysis of steroids in beef.

EXPERIMENTAL

A Shimadzu LCMS-8040 triple quadrupole mass spectrometer was coupled to a Shimadzu Nexera X2 (Shimadzu Scientific Instruments, USA). Steroid standards were obtained from MilliporeSigma (St. Louis, MO), and Cerilliant (Round Rock, Texas). Methanol (LC-MS grade), Acetonitrile (HPLC grade), acetic acid, and ammonium formate were purchased from Millipore Sigma (Burlington, MA). Nanopure water was used. Supel QuE Acetate QuEChERS salt was obtained from Supelco (Bellefonte, PA). A reversed phase superficially porous particle column from Advanced Materials Technology, Inc. (Wilmington, DE) was used; HALO 90 Å C18, 2.7 micron (µm), 2.1 × 100 mm.

Two ground beef samples were procured from the local market, one sample was organic (OGB) and the other was non organic (NOGB).

The steroid panel consisted of Estradiol 17ß, testosterone, progesterone, zeranol, melengestrol acetate (MGA), Aldosterone, corticosterone, and 17 A-methyltestosterone. TBA was not able to be acquired by the lab due to DEA licensing requirements.

SAMPLE PREPARATION

A modified QuEChERS method was used for the extraction of the meat samples. 1.8 grams of OGB and NOGB were vortexed for 2 minutes with 5 mL of ACN, then mixed with 6 grams of Supel QuE Acetate QuEChERS salt, vortexed for 2 minutes then centrifuged at 3500 g for 10 minutes. The ACN level was then filtered via SPE and, following evaporation down to a volume of 5 µL, reconstituted with MEOH to a volume of 100 µL.

Gradient and Mobile Phase

Analytical Column: HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-602
Mobile Phase A: Water, 5 mM Ammonium Formate, 0.1 % Formic Acid, pH 4.0
Mobile Phase B: Methanol
Flow Rate: 0.3 mL/min
Pressure: 190 bar
Temperature: 50 °C
Injection Volume: 2.0µL
Sample Solvent: 45/55/ MEOH/H2O
Detection: +ESI/ -ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS system: Shimadzu LCMS-8040

Gradient:

TIME	%B
0.0	0
2.0	14
3.0	60
3.5	60
8	100
10	100
10.50	0
12.50	Stop

MS Source Conditions:
Spray Voltage: 3.0 kV
Nebulizing gas: 2 L/min
Drying gas: 15 L/min
DL temp: 250 ˚C
Heat Block: 400 ˚C

RESULTS

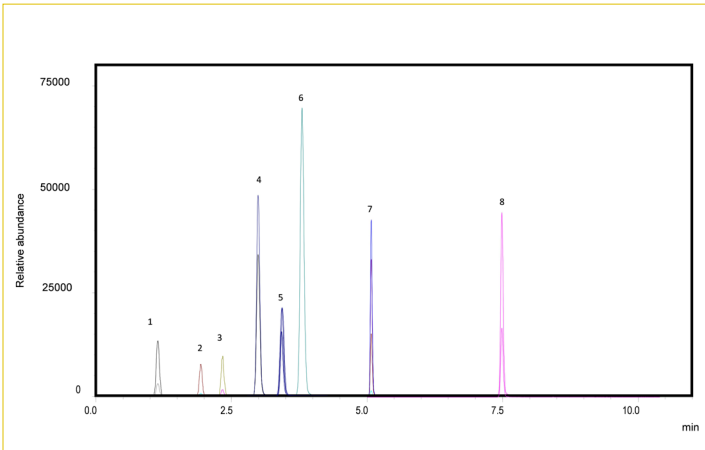


Figure 1. Steroid standards of 10 ng/ml run on HALO® C18

In **Figure 1**, a standard panel of steroids is run on the HALO 90 Å C18, showing a highly resolved separation of all compounds. The panel consisted of common growth promotors and those used for therapeutic purposes, and was chosen to represent the most common steroids that can be expected to be found in beef, through therapeutic or growth promotion utilization. The panel was screened in two different samples of ground beef, an organic and a non-organic brand. The steroids consisted of Estradiol 17ß, testosterone, progesterone, zeranol, melengestrol acetate (MGA), Aldosterone, corticosterone, and 17 A-methyltestosterone.

Peak#	*Compound*	*Transition*	*RT (Min)*
1	*ALDOSTERONE*	*361.0000>343.1000*	*1.154*
2	*CORTICOSTERONE*	*347.6000>109.0000*	*1.965*
3	*ZERANOL*	*321.0000>277.0000*	*2.355*
4	*MGA*	*395.0000> 325.1000*	*3.100*
5	*TESTOSTERONE*	*289.0000>109.0000*	*3.366*
6	*17A-METHYLTESTOSTERONE*	*303.1000>97.0000*	*3.839*
7	*PROGESTERONE*	*315.0000>109.1000*	*5.085*
8	*ESTRADIOL 17ß*	*272.4000>159.1000*	*7.501*

This high-speed separation was achieved in under 8 minutes with high sensitivity at a concentration of 10 ng/mL for meat standards.

Limit of Quantitation

Beef is a very challenging matrix to work with due to the challenges it presents for ionization. Salts, lipids and various components can often suppress ionization; therefore, a successful extraction procedure is paramount to success. In order to determine the effect of the matrix on ionization and determine a rudimentary level of quantitation, both the OGB and the NOGB were spiked with the standards. **Figure 2**, *shows the spiked standards in the OGB sample, in which a level of quantitation was determined down to 1ng/mL.*

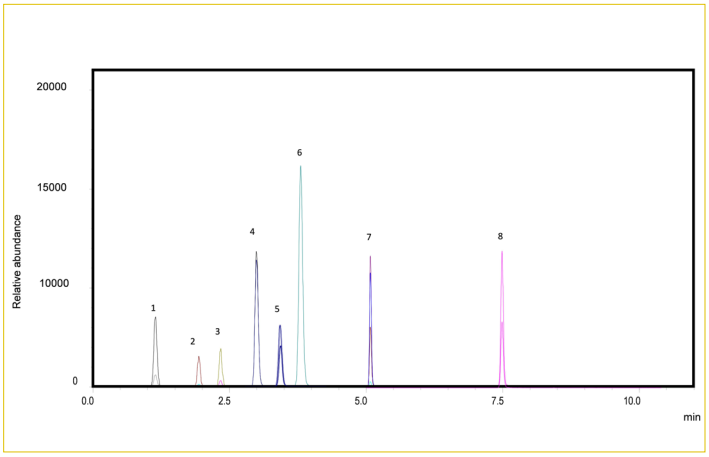


Figure 2. Spiked standard mix in OGB at a level of 1 ng/mL.

In **Figure 3**, this LOQ was determined by performing a calibration curve in which all compounds in the spiked sample could be reliably quantitated.

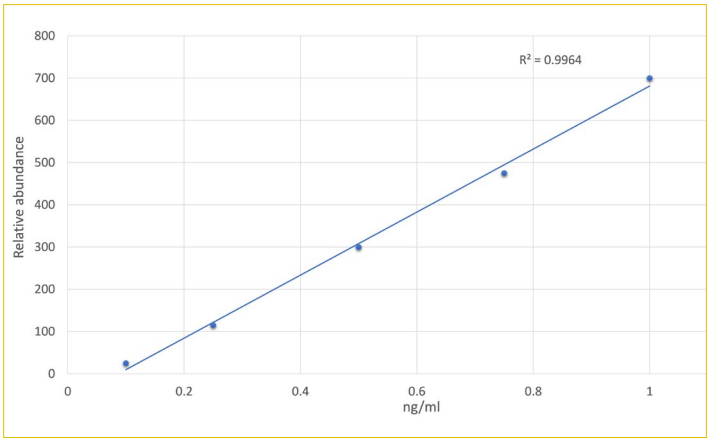


Figure 3. LOQ for steroid mixture of 8 steroids in the spiked OGB sample.

Beef Samples

Once the LOQ had been established for the instrument and QuEChERS extraction, the ground beef samples were extracted. After extraction, both OGB and NOGB samples were analyzed. The samples were screened for the compounds in the standard mix, however; the only detectable steroid in both beef samples was progesterone as shown in (**Figure 4**) for the OGB and (**Figure 5**) for the NOGB.

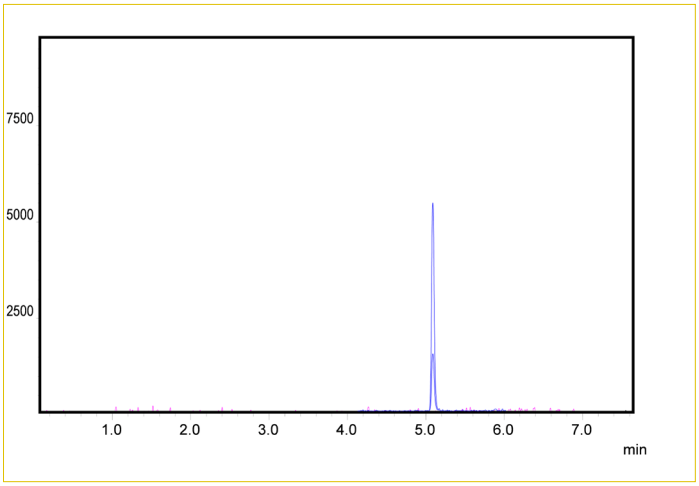


Figure 4. Progesterone detected in OGB at 60 ng/mL

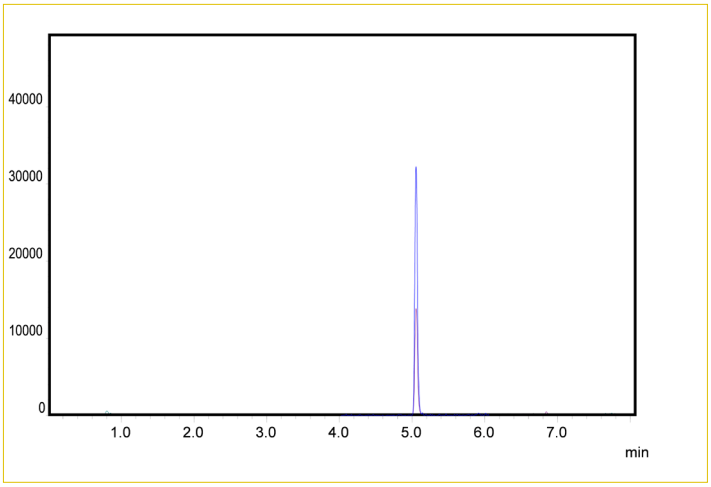


Figure 5. Progesterone in NOGB at 381 ng/mL

A calibration curve (**Figure 6**) was prepared to determine the levels of progesterone in the samples and it was determined that the OGB contained 60 ng/mL of progesterone, while the NOGB contained 381 ng/mL of progesterone. This level is more than 6x the amount found in the OGB, and although progesterone is a naturally occurring steroid found in cattle, this high level is reason for concern. Progesterone has major effects on various bodily systems, and increased research has shown a potential link to exposure of excessively high levels of sex hormones to cancer.[5-11]

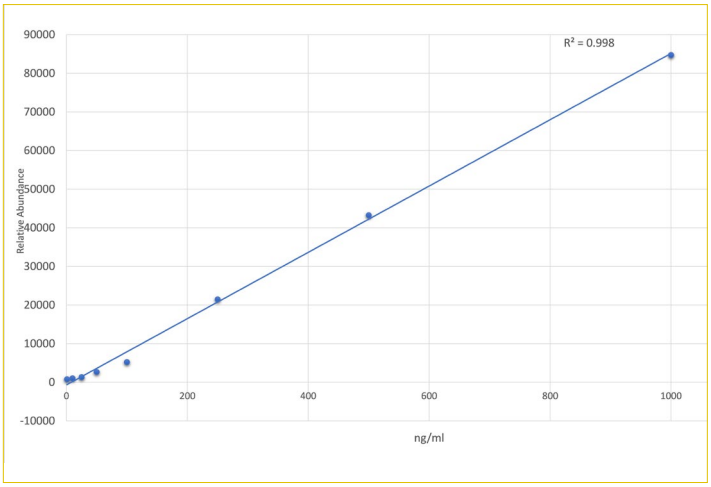


Figure 6. Calibration curve for progesterone

CONCLUSION

The HALO 90 Å C18 column separated steroids in both meat standards and beef with high resolution and high speed, proving to be an ideal choice for this analysis. Despite the challenges presented from beef analysis, particularly the matrix effects, the HALO 90 Å C18 provided the high efficiency and robust performance needed for difficult matrix analysis. Increased exposure to high levels of progesterone, and other sex hormones, has been linked to breast cancer, and actually accelerate tumor growth in these regions.[11-16] This is concerning due to the much higher levels observed in the NOGB, and further regulation is needed to assess and mitigate the potential risks that this presents.

REFERENCES:

1. Fritsche S, Steinhart H. Differences in natural steroid hormone patterns of beef from bulls and steers. J Anim Sci. 1998;76:1621–162 https://www.fda.gov/animal-veterinary/product-safety-information/steroid-hormone-implants-used-growth-food-producing-animals accessed may 2021
2. Font-i-Furnols M., Guerrero L. Consumer preference, behavior and perception about meat and meat products: an overview. Meat Science. 2014;98(3):361–371
3. Troy D. Modern approaches to enhancing beef quality. In: Mesa T., editor. Proceedings of the International 56th Meat Industry Conference; 2011; Tara, Serbia. Tehnologija Mesa; pp. 15–21. http://www.ecfr.gov/. Accessed May 2021
4. Braekevelt E, Lau BP, Tague B, Popovic S, Tittlemier SA. Effect of cooking on concentrations of 17β-estradiol and metabolites in model matrices and beef. J Agric Food Chem. 2011 Feb 9;59(3):915-20.
5. Stephany RW. Hormones in meat: different approaches in the EU and in the USA. APMIS Suppl. 2001;(103):S357-63; discussion S363-4.
6. Directive 2003/74/EC of the European Parliament and of the Council of 22 September 2003 amending Council Directive 96/22/EC concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of beta-agonists Official Journal L 262 , 14/10/2003 P. 0017 – 0021
7. Verbeke W., Van Wezemael L., de Barcellos M. D., et al. European beef consumers' interest in a beef eating-quality guarantee. Insights from a qualitative study in four EU countries. Appetite. 2010;54(2):289–296.
8. Stephany RW. Hormones in meat: different approaches in the EU and in the USA. APMIS Suppl. 2001;(103):S357-63; discussion S363-4.
9. Söderqvist G. Effects of sex steroids on proliferation in normal mammary tissue. Ann Med. 1998;30(6):511-524.
10. Missmer SA, Eliassen AH, Barbieri RL, Hankinson SE. Endogenous estrogen, androgen, and progesterone concentrations and breast cancer risk among postmenopausal women. J Natl Cancer Inst. 2004;96(24):1856-1865.
11. Wiebe JP, Rivas MA, Mercogliano MF, Elizalde PV, Schillaci R. Progesterone-induced stimulation of mammary tumorigenesis is due to the progesterone metabolite, 5α-dihydroprogesterone (5αP) and can be suppressed by the 5α-reductase inhibitor, finasteride. J Steroid Biochem Mol Biol. 2015;149:27-34.
12. Trabert B, Bauer DC, Buist DSM, et al. Association of circulating progesterone with breast cancer risk among postmenopausal women. JAMA Netw Open. 2020;3(4):e203645.
13. M Chen, V Vijay, Q Shi, Z Liu, H Fang, W Tong. FDA-Approved Drug Labeling for the Study of Drug-Induced Liver Injury, Drug Discovery Today, 16(15-16):697-703, 2011.
14. M Chen, A Suzuki, S Thakkar, K Yu, C Hu, W Tong. DILIrank: the largest reference drug list ranked by the risk for developing drug-induced liver injury in humans. Drug Discov Today 2016, 21(4): 648-653
15. DHHS/National Toxicology Program; Eleventh Report on Carcinogens: Progesterone (57-83-0) (January 2005)