Application Note

Food & Beverage Testing

Determination of 58 Glucocorticoids in Milk

Using Agilent Captiva EMR–Lipid HF passthrough cleanup and LC/MS/MS detection

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Abstract

This application note presents the development and validation of a multiresidue method for the analysis of 58 glucocorticoids in milk. The method uses modified QuEChERS extraction, followed by enhanced matrix removal (EMR) passthrough cleanup with the Agilent Captiva EMR–Lipid HF cartridges, then LC/MS/MS detection. The use of Captiva EMR–Lipid HF cartridges makes complex food sample extract elution on gravity easier and quicker, without compromise of matrix removal or target recovery. The method was demonstrated to meet the required limits of quantitation (LOQs), recovery, and repeatability for glucocorticoid targets in cow milk and goat milk.

Introduction

Determining glucocorticoid residues in foods of animal origin is important for meeting global food safety requirements. China's National Food Safety Standard GB 31650-2019[1] sets maximum residue limits (MRLs) for betamethasone and dexamethasone at 0.3 µg/kg in milk; for betamethasone at 0.75 µg/kg in beef, pork, and kidney; for dexamethasone at 1 µg/kg in beef, pork, horsemeat, and kidney, and 2 µg/kg in liver.

The current method for glucocorticoids residue analysis in animal-origin foods has limitations on the number of targets and requires a more complicated sample preparation procedure, which takes longer time and more solvent (> 54 mL organic solvent used).[2] In this study, a method was developed and validated for the analysis of 58 glucocorticoids using new Agilent Captiva EMR–Lipid HF cartridges with LC/MS/MS detection and the Agilent InfinityLab Poroshell 120 EC-C18 column.

Captiva EMR–Lipid HF is a newly developed product using the Captiva EMR–Lipid sorbent chemistry but with improvements in usability for complex fatty food matrices such as meat, fish, oils, etc. Captiva EMR–Lipid HF cartridges allow gravity elution within an acceptable time window (15 to 25 minutes) for most complex and fatty matrices for food of animal origin, and they also deliver consistent elution from cartridge to cartridge. Captiva EMR–Lipid HF cartridges provide equivalent or slightly better matrix removal and equivalent target recovery compared to corresponding current Captiva EMR–Lipid cartridges. The use of Captiva EMR–Lipid HF cartridges is same as Captiva EMR–Lipid cartridges, where crude extract needs to be premixed with 10 to 20% water before loading. For the Captiva EMR–Lipid HF 3 mL format, the typical loading volume is 2.5 to 3 mL, while for the 6 mL format, the typical loading volume is 5 to 6 mL. The only difference is that Captiva EMR–Lipid HF provides better feasibility for sample gravity elution for many typical complex food matrices.

Experimental

Chemicals and reagents

Glucocorticoids stock solutions were purchased from ChemicalBook (Changzhou, China). Cow milk and goat milk were purchased from local grocery store.

The individual stock solutions were prepared at 1,000 µg/mL in methanol (MeOH). The combined spiking solution was then prepared by diluting individual stock solutions to 10 µg/mL in acetonitrile (ACN) and storing in a freezer at –20 °C.

Matrix-matched calibration curve standards were prepared using cow milk and goat milk matrix blanks at 0.1, 0.2, 0.5, 1.0, 5.0, and 20 ng/mL for 47 targets with required LOQs at 0.2 ng/mL and at 1.0, 2.0, 5.0, 10, 20, and 50 ng/mL for the other 11 targets with required LOQs at 2.0 ng/mL.

Equipment and material

The study was performed using an Agilent 1290 Infinity II LC system coupled to the Agilent 6470B triple quadrupole LC/MS. Agilent MassHunter Workstation software was used for data acquisition and analysis.

Chromatographic separation was performed using an Agilent InfinityLab Poroshell 120 EC-C18 column, 3.0 × 100 mm, 2.7 µm (part number 695975-302).

Other Agilent consumables used included:

- Agilent Bond Elut QuEChERS extraction kit for vet drugs (part number 5982-0032 (50/pk) or 5982-6032 (no centrifuge tubes, 50/pk))
- Captiva EMR–Lipid HF cartridges, 6 mL cartridges (part number 5610-2236)
- Agilent ValueLab filter, hydrophilic, PTFE, 0.2 µm, 13 mm (part number 5191-4294)
- Agilent ceramic homogenizers, 50 mL tubes (part number 5982-9313)

LC/MS/MS instrument conditions

The LC pump conditions are listed in Table 1, and the mass spectrometer method conditions on the Agilent 6470B triple quadrupole LC/MS are listed in Table 2.

The ESI source settings include drying gas at 300 °C, 7 L/min; sheath gas at 350 °C, 12 L/min; nebulizer gas at 35 psi; capillary voltage at 3,500 V; and acquisition mode at dMRM ESI (+). The targets MRM transition settings are listed in Table 2.

Table 1. LC method conditions for LC/MS/MS.

Parameter	Setting			
Mobile Phase A	Water with 0.1% formic acid and 1 mmol/L ammonium fluoride			
Mobile Phase B	ACN			
Column Temperature	40 °C			
Injection Volume	10 µL			
Gradient	Time (min)	%A	%B	Flow (mL/min)
	0.00	75.00	25.00	0.500
	8.00	63.00	37.00	0.500
	18.00	20.00	80.00	0.500
	20.00	0.00	100.00	0.500
Post Time	3.0 min			

Table 2. LC/MS/MS acquisition settings (continued on next page).

Compound	Retention Time (min)	Precursor Ion (*m/z*)	Fragmentor (V)	Product Ions (*m/z*) [Collision Energy (V)]	
				Quantifier Ion	Qualifier Ion
Triamcinolone	2.36	395.2	140	357.1 [8]	225.1 [14]
Prednisolone	3.90	361.2	110	343.1 [6]	146.9 [20]
Isoflupredone	3.91	359.2	115	359.1 [6]	341.1 [10]
Prednisone	3.91	359.2	90	341.1 [6]	147 [24]
Hydrocortisone	4.08	363.2	130	121.0 [24]	105.1 [50]
Cortisone	4.20	361.2	120	163.1 [20]	121 [30]
Methylprednisolone	5.62	375	120	357.0 [10]	161 [18]
Betamethasone	5.90	393.2	110	373.3 [6]	355 [10]
Flumethasone	6.07	393.2	110	121.1 [10]	253 [34]
Beclomethasone	6.11	411.2	120	391.1 [6]	146.9 [30]
Cloprednol	6.63	409.2	110	205.0 [30]	271 [25]
Triamcinolone Acetonide	6.78	393.2	120	396.9 [10]	338.9 [10]
Desonide	7.06	435.2	110	399.2 [10]	323.2 [10]
Flunisolide	7.08	417.2	140	339.2 [10]	321.1 [10]
Triamcinolone Acetonide Diacetate	7.28	435.2	125	321.0 [10]	440.9 [4]
Fluocinolone	7.62	479.2	140	121.1 [10]	413.2 [40]
Fludroxycortide	7.69	453.2	120	120.8 [30]	180.9 [40]
Prednisolone Acetate	7.74	437.2	160	295.0 [8]	146.8 [24]
Hydrocortisone Acetate	7.79	403.2	110	309.1 [12]	120.8 [34]
Fluorometholone	8.14	405.2	144	278.9 [10]	320.9 [8]
Fludrocortisone Acetate	8.25	377.2	110	238.9 [22]	120.9 [36]
Prednisone Acetate	8.28	423.2	160	295.0 [8]	146.8 [24]
Deflazacort	8.68	401.2	120	141.9 [36]	123.9 [50]
Cortisone Acetate	8.90	442.2	180	162.8 [16]	343 [24]
Halometasone	9.10	403.3	160	155.0 [40]	169 [34]
Methylprednisolone Acetate	9.14	445.2	120	399.2 [6]	339.2 [10]
Betamethasone Acetate	9.99	417.2	110	337.0 [8]	309 [8]
Paramethasone Acetate	10.06	435.2	110	337.2 [10]	417 [10]
Budesonide	10.44	435.2	125	413.1 [6]	146.9 [30]
Dexamethasone Acetate	10.59	431.2	110	337.0 [8]	309 [8]
Hydrocortisone Butyrate	10.59	435.2	110	120.8 [24]	345 [8]
Fluorometholone Acetate	10.74	433.2	140	279.0 [10]	321 [8]

Table 2. LC/MS/MS acquisition settings (continued from previous page).

Compound	Retention Time (min)	Precursor Ion (*m/z*)	Fragmentor (V)	Product Ions (*m/z*) [Collision Energy (V)]	
				Quantifier Ion	Qualifier Ion
Triamcinolone Acetonide Acetate	11.13	419.2	110	338.9 [10]	320.8 [12]
Medrysone	12.11	477.2	110	43.2 [50]	97.1 [38]
Hydrocortisone Valerate	12.18	345.2	170	345.2 [8]	120.8 [30]
Fluocinolone Acetate	12.17	447.3	130	121.0 [40]	475.2 [12]
Diflurasone Acetate	12.36	495.2	130	316.8 [8]	278.8 [10]
Hydrocortisone Aceponate	12.39	495.2	120	43.2 [50]	387.2 [10]
Betamethasone-17a-Valerate	12.58	461.3	155	278.8 [14]	354.9 [4]
Difluprednate	13.14	477.3	110	43.2 [50]	303.1 [14]
Methylprednisolone Aceponate	13.16	509.2	130	455.2 [6]	381 [13]
Halcinonide	13.33	473.3	125	121.1 [41]	104.8 [61]
Prednicarbate	13.59	455.2	160	380.9 [6]	114.8 [12]
Loteprednol Etabonate	13.69	489.2	120	265.1 [18]	359.1 [10]
Amcinonide	13.78	467.2	140	321.0 [14]	338.9 [10]
Alclometasone Dipropionate	13.90	503.2	110	301.0 [10]	279 [10]
Alclometasone Dipropionate	13.94	521.2	130	121.0 [51]	261 [29]
Clobetasol Propionate	13.96	485.2	180	372.9 [6]	354.9 [8]
Tixocortol-21-Pivalate	14.00	467.2	110	57.2 [46]	377.1 [18]
Fluticasone Propionate	14.11	463.2	140	292.9 [10]	312.9 [8]
Mometasone Furoate	14.24	501.2	110	503.0 [4]	263 [24]
Prednisolone Acetate Valerate	14.35	521.1	120	43.2 [50]	101 [38]
Betamethasone Dipropionate	14.32	487.3	125	278.9 [12]	318.9 [10]
Beclomethasone Dipropionate	14.55	505.2	110	57.1 [22]	503.1 [12]
Betamethasone Butyrate Propionate	15.13	521.2	135	57.2 [50]	319.1 [14]
Difluocortolone Valerate	15.46	519.3	140	57.2 [50]	85.1 [22]
Clobetazone Butyrate	15.48	479.3	130	278.9 [14]	342.8 [12]

Sample preparation

Cow milk and goat milk were purchased from a local grocery store. Samples were then ready for the procedure described in Figure 1.

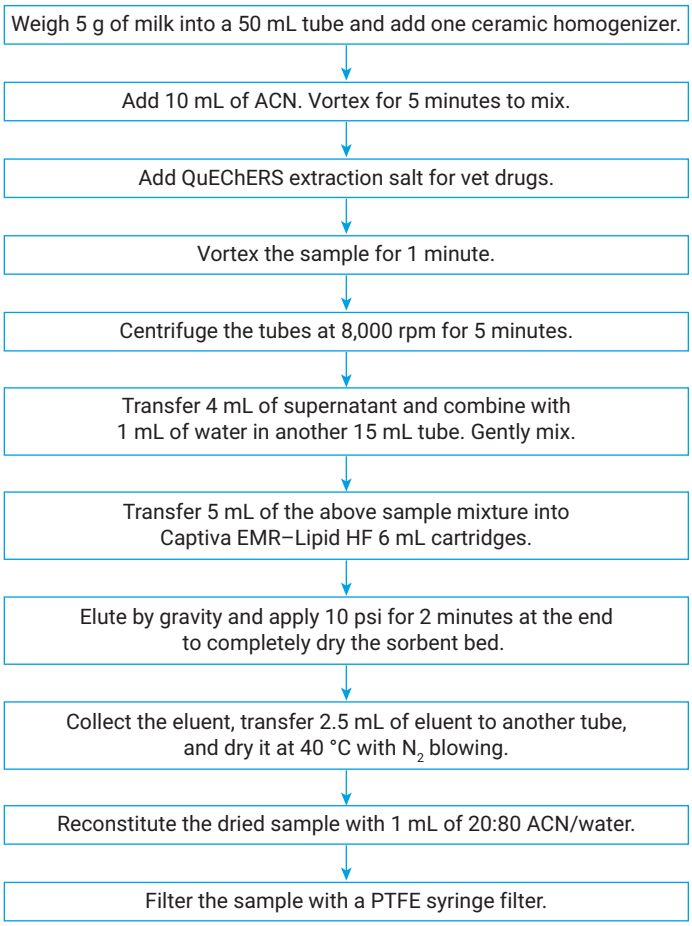

Figure 1. Sample preparation procedure for glucocorticoid analysis in milk.

Results and discussion

Captiva EMR–Lipid HF cartridges were developed to improve the cartridge design and the feasibility for gravity elution for complex food matrix extracts. Especially for fatty food matrices such as meat, fish, eggs, milk, and oils, sample crude extracts loaded on the cartridge can flow through by gravity within an acceptable time range (10 to 25 minutes). To compare the sample elution on Captiva EMR–Lipid HF cartridges, the currently available commercial products, and major competition products, eight representative food matrices were used for crude sample extract gravity elution. Table 3 shows the sample elution time needed for equivalent crude sample extract elution by gravity on three types of cartridges with the same tube size and corresponding sorbent bed mass, using the same sample extract with equivalent loading volume. The results showed that Captiva EMR–Lipid HF cartridges provided sample elution by gravity within 25 minutes for all food matrices except bovine kidney, with which took slightly longer than 25 minutes to elute due to significant sample complexity. Comparing to other comparable cartridges, the elution time was shortened by 20 to 50%.

Table 3. Complex food matrix extract gravity elution time comparison using different cartridges for matrix passthrough cleanup.

Food Matrix Extract	Sample Extract Gravity Elution Time (min)		
	Agilent Captiva EMR–Lipid HF	Current Cartridge	Competition Cartridge
Beef	18 to 22	45 to 47	38 to 46
Pork	22 to 24	41 to 45	32 to 47
Bovine Kidney	22 to 26	48 to 51	31 to 54
Salmon	15 to 20	36 to 40	19 to 26
Eggs	11 to 15	23 to 25	34 to 37
Infant Formula	12 to 14	15 to 17	10 to 12
Chocolate	12 to 14	30 to 37	20 to 74
Peanut Oil	13 to 17	19 to 22	74 to 76
Pumpkin Seed Oil	20 to 25	23 to 25	> 90

The matrix removal and target recovery were also assessed and compared for Captiva EMR–Lipid HF cartridges. For matrix removal evaluation, the cartridge performance was evaluated by using a sample co-extractives dry residue method and a GC/MS full scan chromatographic background screening using the eight types of fatty food matrices. The samples were extracted appropriately, then cleaned using different cleanup cartridges. The cleaned sample extract was compared to the crude extract before cleanup. Figure 2 shows the matrix removal comparison results, demonstrating that the use of Captiva EMR–Lipid HF provided equivalent or even slightly better matrix removal than current and competition products. The target recovery was evaluated and compared with the current product for 58 targets in this study. The results shown in Figure 3 confirms the equivalence of target recovery when using Captiva EMR–Lipid HF cartridges and current products.

The cartridge performance evaluation study confirmed that Captiva EMR–Lipid HF cartridges provide improved usability and feasibility for sample elution by gravity without impact on the matrix removal and target recovery.

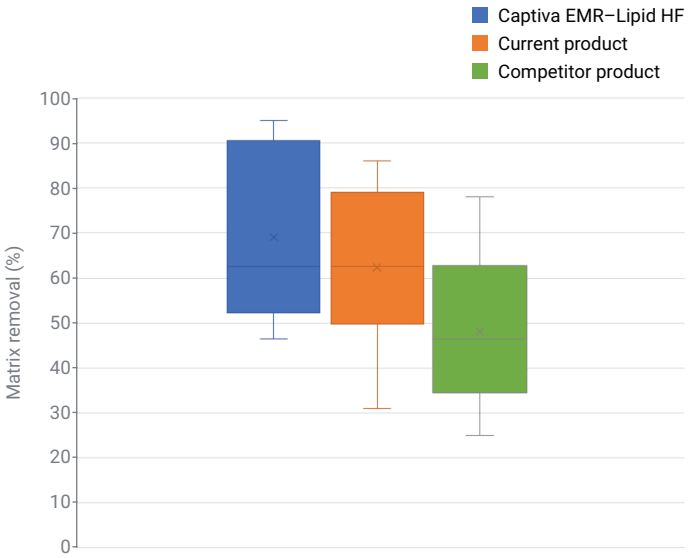

Figure 2. Matrix removal comparison for eight fatty food matrices using Captiva EMR–Lipid HF cartridges, current products, and competition products.

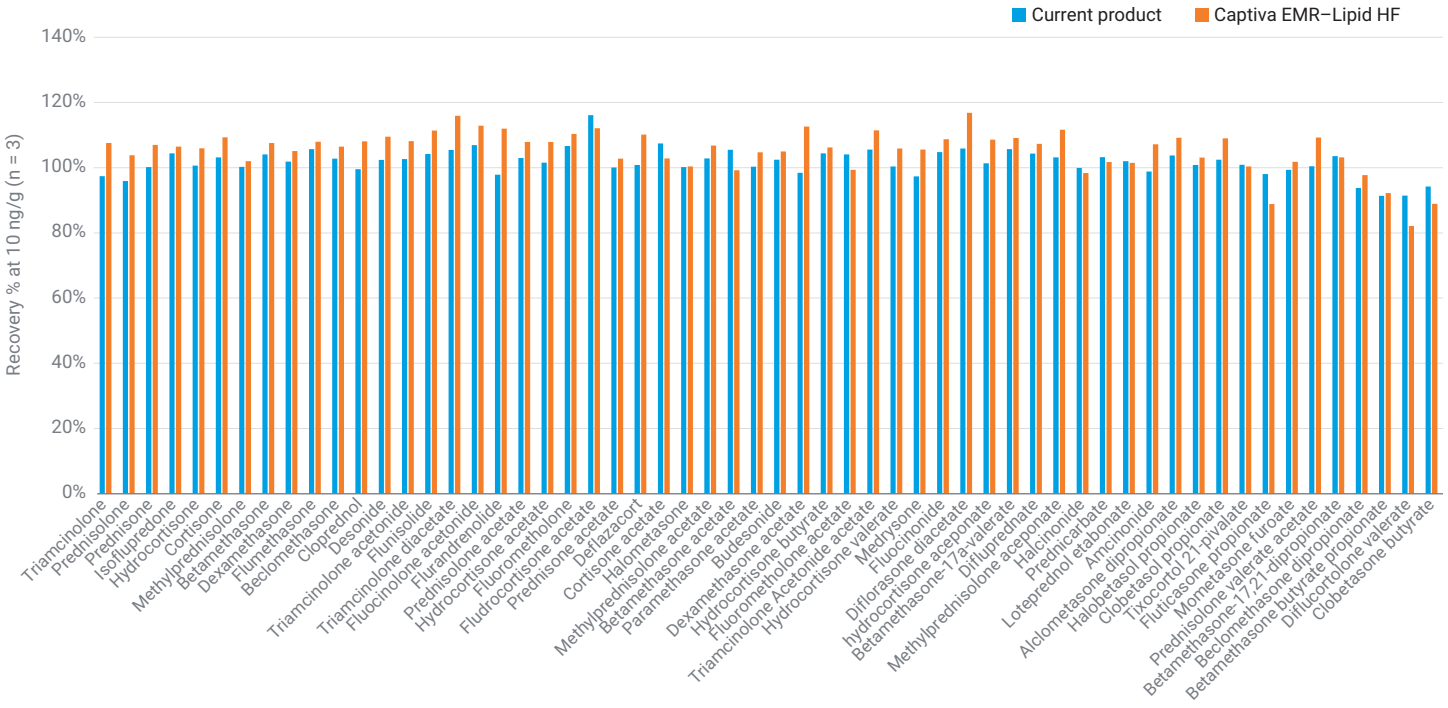

Figure 3. Target recovery comparison for 58 glucocorticoids in milk using Agilent Captiva EMR–Lipid HF cartridges and current products.

Mobile phases selection

The original mobile phases used in the study, water with 0.1% formic acid (FA) and ACN, did not provide acceptable method sensitivity for 58 glucocorticoids. The mobile phase additive ammonium fluoride (NH_4F) was added to improve the compound ionization efficiency, and thus the targets' responses on LC/MS/MS. Figure 4 shows the chromatographic comparison between using water with 0.1% FA (black) as mobile phase A and water with 0.1% FA and 1 mmol/L NH_4F (red). The results clearly demonstrate that the glucocorticoids target responses were improved significantly when using a mobile phase of water with 1% FA and 1 mmol/L NH_4F, and this mobile phase was chosen for the optimized method.

Sample matrix cleanup method comparison

The sample matrix cleanup method using Agilent Captiva EMR–Lipid HF cartridges was compared with traditional dispersive SPE (dSPE) cleanup using the Agilent Bond Elut QuEChERS dSPE kit for vet drugs (part number 5982-4950) for target recovery. The target recovery comparison results (Figure 5) showed that improved recovery was achieved when using Captiva EMR–Lipid HF for sample matrix cleanup. The recovery for all targets was demonstrated within 70 to 120% using Captiva EMR–Lipid HF passthrough cleanup, while traditional dSPE cleanup caused the loss of many targets, especially intermediate to highly hydrophobic compounds.

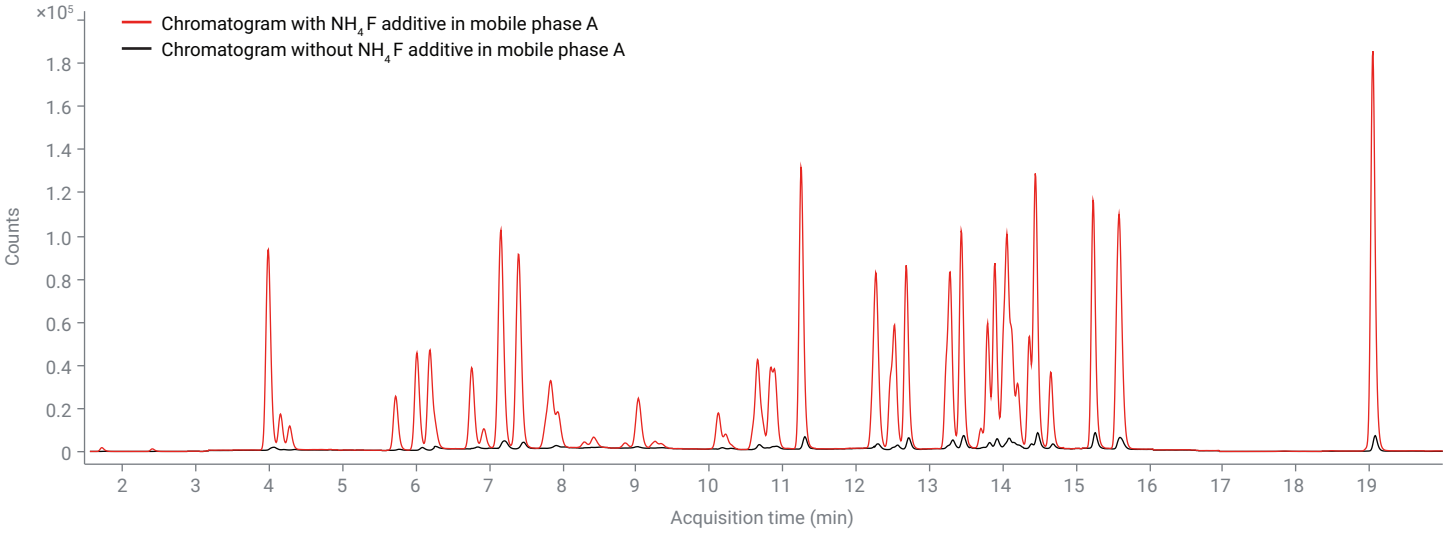

Figure 4. Chromatograms of 58 glucocorticoids using mobile phase A with (red) and without (black) 1 mmol/L NH_4F.

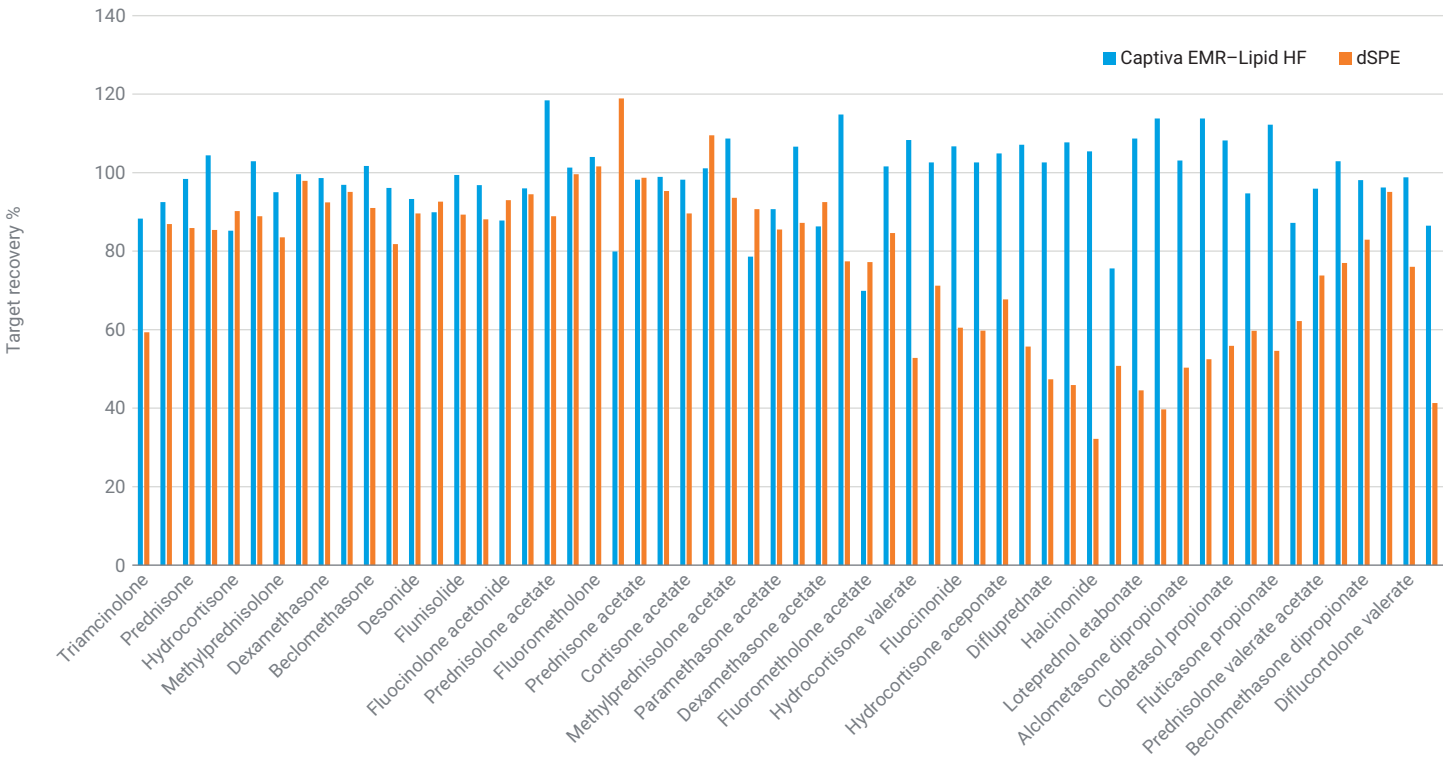

Figure 5. Recovery of 58 glucocorticoids using Captiva EMR–Lipid HF passthrough cleanup and traditional dSPE cleanup.

Calibration standards curve

A matrix-matched calibration standard curve was prepared by spiking standards appropriately into matrix blanks prepared using the developed method. For 47 targets with required LOQs at 0.2 ng/mL, the matrix-matched calibration standards included 0.1, 0.2, 0.5, 1.0, 5.0, and 20 ng/mL in the matrix blanks. For the remaining 11 targets with required LOQs at 2 ng/mL, the matrix-matched calibration standards included 1.0, 2.0, 5.0, 10, 20, and 50 ng/mL in the matrix blanks. Results confirmed all the target matrix-matched calibration curves delivered acceptable linearity with $R^2 > 0.99$.

Method quantitation results

Table 4 shows the method validation results for the quantitative determination of 58 glucocorticoids in cow milk. The results demonstrate acceptable method LOQs (0.2/2 µg/kg), recovery (90 to 109%) and RSD (1.2 to 14.0%), which meet the GB regulation.

Table 4. Method validation results for quantitative determination of 58 glucocorticoids in cow milk (continued on next page).

Target	LOQ (µg/kg)	Matrix Spiking Concentration (n = 6 at each level)					
		0.2/2 µg/kg		1/5 µg/kg		2/10 µg/kg	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Triamcinolone	2	91.4	10.8	93.7	9.4	107.5	4.8
Prednisolone	0.2	100.2	8.9	104.7	7.3	103.8	2.3
Isoflupredone	0.2	100.4	7.0	101.4	8.7	107.0	1.4
Prednisone	0.2	97.4	6.6	101.5	8.8	106.5	1.0
Hydrocortisone	0.2	97.7	5.2	101.4	9.0	105.9	1.3
Cortisone	0.2	101.6	7.1	98.2	7.1	109.0	2.8
Methylprednisolone	0.2	100.7	4.6	100.2	7.7	102.0	2.0
Betamethasone	0.2	105.9	8.5	97.4	5.2	107.5	2.5
Flumethasone	0.2	96.7	8.7	98.2	9.5	105.1	1.5
Beclomethasone	0.2	97.7	6.4	97.2	5.6	108.0	1.5
Cloprednol	0.2	103.7	2.1	105.7	5.2	106.4	1.1
Triamcinolone Acetonide	0.2	101.4	5.7	100.2	9.3	108.1	2.8
Desonide	0.2	104.7	6.7	104.7	8.0	109.5	1.3
Flunisolide	0.2	100.6	7.0	99.4	9.1	108.2	3.1
Triamcinolone Acetonide Diacetate	0.2	99.4	5.9	102.6	6.4	111.4	1.8
Fluocinolone	0.2	103.0	6.5	102.7	6.4	115.9	0.7
Fludroxycortide	0.2	102.2	7.6	97.9	9.4	112.9	1.4
Prednisolone Acetate	2	90.1	13.7	92.9	6.9	112.0	3.8
Hydrocortisone Acetate	0.2	105.6	6.9	100.7	9.8	107.9	3.7
Fluorometholone	2	104.2	7.0	104.0	6.8	107.9	3.5
Fludrocortisone Acetate	2	92.7	12.3	95.1	9.7	110.4	5.4
Prednisone Acetate	2	92.6	11.2	93.1	7.9	112.1	4.1
Deflazacort	2	101.2	6.6	101.4	7.5	102.8	5.5
Cortisone Acetate	0.2	102.4	3.6	98.9	6.6	110.2	3.0
Halometasone	2	97.7	7.2	98.1	7.5	102.8	4.4
Methylprednisolone Acetate	2	101.9	7.7	102.6	6.2	100.4	2.3
Betamethasone Acetate	0.2	102.7	5.1	103.4	8.4	106.8	1.3
Paramethasone Acetate	2	103.7	8.6	105.6	6.0	99.2	3.9
Budesonide	2	100.4	6.3	102.6	9.5	104.7	6.6
Dexamethasone Acetate	0.2	97.7	8.2	96.5	7.0	104.9	1.2
Hydrocortisone Butyrate	2	98.4	9.9	97.7	11.7	112.6	1.3
Fluorometholone Acetate	0.2	99.4	6.2	101.2	8.0	106.2	0.3
Triamcinolone Acetonide Acetate	0.2	99.4	8.3	107.1	4.5	99.3	2.7
Medrysone	0.2	103.6	7.2	101.2	5.8	111.4	1.8
Hydrocortisone Valerate	0.2	106.5	4.3	101.9	8.5	105.9	1.5
Fluocinolone Acetate	0.2	95.2	5.8	103.4	5.5	105.5	1.3
Diflurasone Acetate	0.2	104.4	7.6	105.6	4.8	108.7	0.7
Hydrocortisone Aceponate	0.2	99.4	9.4	98.1	5.1	116.8	1.7
Betamethasone-17a-Valerate	0.2	107.1	6.9	101.4	7.7	108.6	1.2
Difluprednate	0.2	102.1	5.1	103.4	6.9	109.1	1.5
Methylprednisolone Aceponate	0.2	103.7	7.8	98.4	7.4	107.3	0.5
Halcinonide	0.2	107.2	7.7	102.4	6.3	111.7	1.3
Prednicarbate	0.2	98.4	5.3	97.7	5.6	98.3	1.6
Loteprednol Etabonate	0.2	97.0	9.7	100.0	6.8	101.7	1.0

Table 4. Method validation results for quantitative determination of 58 glucocorticoids in cow milk (continued from previous page).

Target	LOQ (µg/kg)	Matrix Spiking Concentration (n = 6 at each level)					
		0.2/2 µg/kg		1/5 µg/kg		2/10 µg/kg	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Amcinonide	0.2	105.6	7.7	99.2	7.4	101.5	1.3
Alclometasone Dipropionate	0.2	98.7	7.4	100.4	5.8	107.2	2.8
Alclometasone Dipropionate	0.2	100.7	8.3	101.2	7.5	109.2	1.2
Clobetasol Propionate	0.2	102.7	5.1	95.7	4.6	103.1	4.1
Tixocortol-21-Pivalate	0.2	101.4	8.0	100.9	6.2	109.0	1.5
Fluticasone Propionate	0.2	95.7	7.1	108.9	1.2	100.3	0.8
Mometasone Furoate	0.2	103.1	7.1	99.2	8.2	88.9	1.0
Prednisolone Acetate Valerate	0.2	107.4	7.6	98.4	8.0	101.8	1.2
Betamethasone Dipropionate	0.2	100.2	7.6	104.9	4.7	109.2	1.5
Beclomethasone Dipropionate	0.2	103.2	6.6	102.4	8.6	103.2	3.1
Betamethasone Butyrate Propionate	0.2	103.1	8.7	97.4	7.4	97.7	1.2
Difluocortolone Valerate	0.2	104.0	8.4	105.4	4.9	92.2	1.0
Clobetazone Butyrate	0.2	95.9	5.1	105.1	7.5	82.1	1.6
Triamcinolone	0.2	103.2	5.5	103.2	4.8	88.9	2.5

Conclusion

A simplified, rapid, and reliable method using QuEChERS extraction followed by Agilent Captiva EMR–Lipid HF passthrough cleanup was developed and validated for 58 glucocorticoids in cow milk and goat milk. The new Captiva EMR–Lipid HF cartridges demonstrated with improved usability for complex food sample elution under gravity within acceptable elution time window, without compromise in matrix removal and target recovery. The validated method delivers acceptable LOQs at 0.2 µg/kg for 47 targets and 2 µg/kg for 11 targets, recovery (90 to 109%) and RSD (1.2 to 14.0%) for 58 glucocorticoids in cow milk and goat milk.

References

1. Food and Agriculture Organization of the United Nations. FAOLEX Database: National Food Safety Standard – Maximum Residue Limit for Veterinary Medicines in Foods (GB 31650—2019). https://faolex.fao.org/docs/pdf/chn215905.pdf
2. China Ministry of Agriculture Announcement No. 1031-2-2008: Determination of Multiresidue Glucocorticoid Drugs in Animal-Origin Foods by LC/MS/MS. http://www.moa.gov.cn/nybgb/2008/dliuq/201806/t20180610_6151596.htm

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