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# Simultaneous determination of seventeen glucocorticoids residues in milk and eggs by ultra-performance liquid chromatography/electrospray tandem mass spectrometry

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**A comprehensive analytical method has been developed and validated for the simultaneous determination of seventeen glucocorticoid residues in eggs and milk. The mass spectrometer parameters, the composition of the mobile phase and the sample preparation method were firstly optimized to obtain maximum sensitivity. The samples were deconjugated with β-glucuronidase/arylsulfatase enzyme and concentrated using an Oasis HLB solid-phase extraction cartridge, followed by cleanup with a dual Sep-pak silica and aminopropyl cartridge. The analytes were quantified by ultra-performance liquid chromatography (using a C18 column)/electrospray ionization tandem mass spectrometry (UPLC/ESI-MS/MS) operating in the negative ion mode. The assay for the 17 glucocorticoids was linear over the range of 1–200 μg/L for milk and egg samples with a high correlation coefficient (>0.99). The limits of quantification (LOQs) for the target analytes were 0.04–1.27 μg/kg for the egg samples and 0.03–0.73 μg/kg for the milk samples. The average extraction recoveries of the glucocorticoids from eggs and milk at two concentration levels (spiked at 0.40 and 2.00 μg/kg) were 65.6–118.7% and 61.5–119.6%, respectively, with relative standard deviations between 1.8–17.0% and 2.4–18.4%, respectively. Because of its high sensitivity, good precision and specificity, the method was found to be suitable for trace analysis of synthetic and natural glucocorticoids in complex biosamples such as eggs and milk.** 

Glucocorticoids are steroid hormones secreted by the suprarenal cortex, and excreted in urine in a non-metabolized form, primarily as conjugated metabolites.[1,2] Glucocorticoids have metabolic and anti-inflammatory properties and help protect against stress and shock. Artificial glucocorticoids have been synthesized and used in many veterinary therapeutic drugs for the treatment of inflammatory diseases.[3–6] These compounds also increase weight gain by means of water and fat retention, and they have a synergetic effect when combined with compounds like β-agonists or anabolic steroids.[7–9] Due to their adverse effects on human health, glucocorticoids are illegal to use as growth promoters in the European Union and China.[10–12] For animals that will be used for human consumption, only dexamethasone, betamethasone, prednisolone and methylprednisolone have been approved for therapeutic use. Maximum residue limits (MRLs) have been established by the European Commsion.[13] The MRLs for both betamethasone and dexamethasone are 2 μg/kg in liver, 0.75 μg/kg in muscle and kidney, and 0.3 μg/kg in milk samples. For methylprednisolone, the MRL is 10 μg/kg in all matrices, but methylprednisolone cannot be used in animals that produce milk for human consumption. The MRLs for prednisolone are 10 μg/kg in liver and kidney, 4 μg/kg in muscle and fat, and 6 μg/kg in milk. China has established a MRL of 10 μg/kg for hydrocortisone in milk, and a MRL of 0.75 μg/kg for dexamethansone in muscle, liver and kidney.

Various separation techniques including gas and liquid chromatography coupled with mass spectrometry have been developed to assay residual levels of glucocorticoids in biological samples.[14–31] Gas chromatography/mass spectrometry (GC/MS) methods provide high sensitivity, specificity and chromatographic resolution; however, these methods require derivatization to enhance the volatility of the analytes.[14–17] Liquid chromatography/mass spectrometry (LC/MS) is also a promising technique for residual

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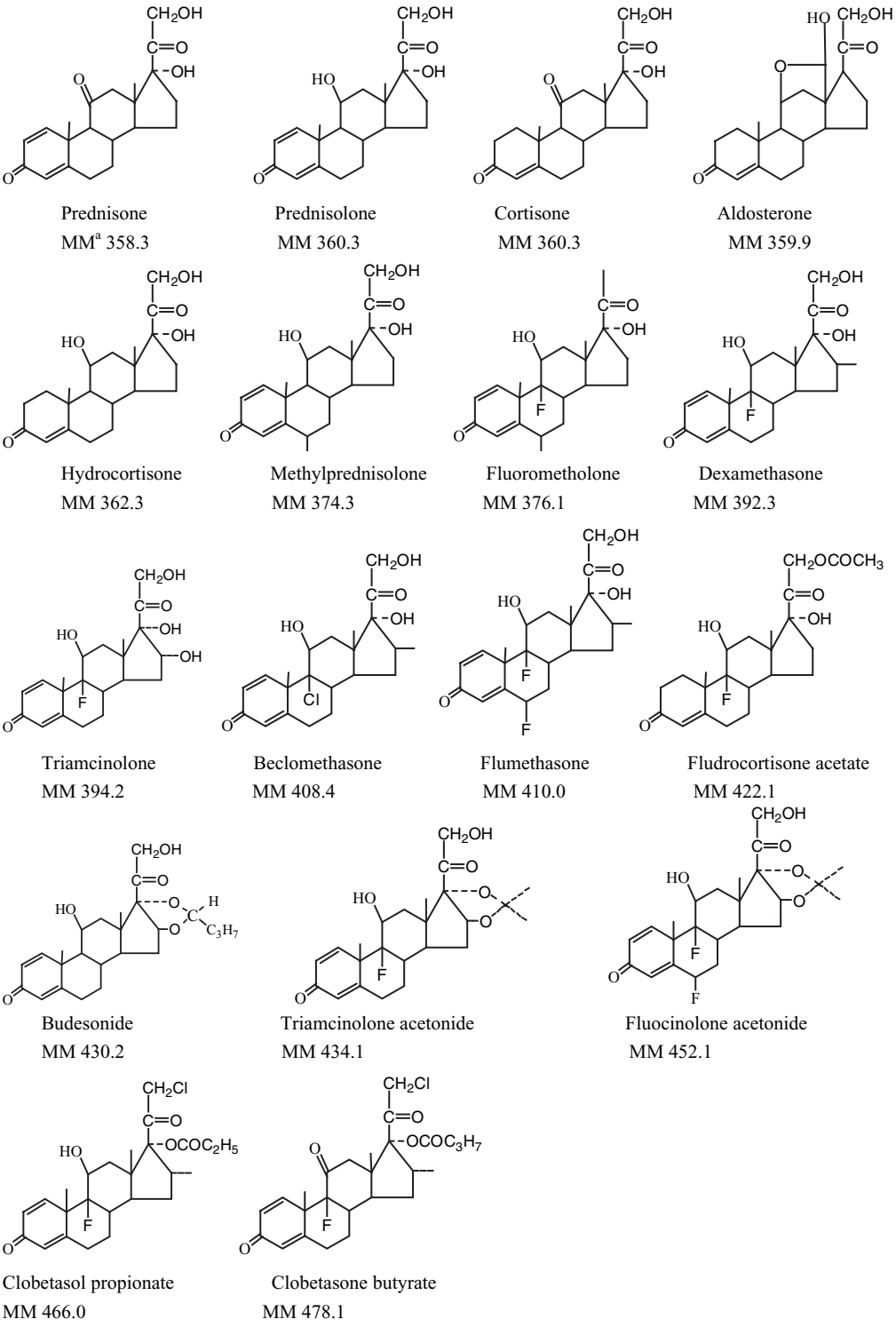


**Figure 1.** Chemical structures of the analyzed glucocorticoids. [a]Monoisotopic molecular weight.

analysis because of its high selectivity, specificity and sensitivity to glucocorticoid residues. There is no need for derivatization steps, which reduces the analysis time, eliminates sources of error and decreases the use of hazardous and expensive reagents. Many LC/MS methods have been developed for measuring glucocorticoid residues,[18–31] but no techniques have been reported for determining residues in egg samples. In the screening and confirmation of residues, the number of target components and the analysis time are top priorities. To the best of our knowledge, no methods have been reported that can simultaneously detect more than twelve glucocorticoid residues in foods of animal origin.

In this study, a rapid analytical method using ultra-performance liquid chromatography/electrospray ionization tandem mass spectrometry (UPLC/ESI-MS/MS) in negative ion mode has been developed for the simultaneous determination of 17 glucocorticoid residues in eggs and milk.





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**Table 1.** LC/MS/MS acquisition parameters for the 17 compounds

| Compound | Retention time (min) | Precursor ion (*m*/*z*) | Product ion[a] (*m*/*z*) | Collision energy (eV) |
|---|---|---|---|---|
| Prednisone | 7.63 | 403.3 | 327.2, 357.2 | 8 |
| Prednisolone | 7.93 | 405.3 | 329.1, 359.0 | 13 |
| Cortisone | 8.62 | 405.3 | 329.1, 359.0 | 8 |
| Aldosterone | 6.61 | 404.9 | 358.9, 331.2 | 9 |
| Hydrocortisone | 8.60 | 407.3 | 330.8, 361.1 | 15 |
| Methylprednisolone | 9.96 | 419.3 | 343.1, 373.1 | 11 |
| Fluorometholone | 10.29 | 421.1 | 355.1, 375.3 | 9 |
| Dexamethasone | 9.80 | 437.3 | 361.1, 391.0 | 12 |
| Triamcinolone | 2.75,4.72 | 439.2 | 363.1, 393.1 | 13 |
| Beclomethasone | 9.99 | 453.4 | 376.8, 406.8 | 14 |
| Flumethasone | 9.52 | 455.0 | 378.7, 409.0 | 15 |
| Fludrocortisone acetate | 9.90 | 467.1 | 421.1, 403.3 | 11 |
| Budesonide | 11.60 | 475.2 | 357.1, 339.1 | 13 |
| Triamcinolone acetonide | 9.90 | 479.1 | 375.0, 356.7 | 19 |
| Fluocinolone acetonide | 10.04 | 497.1 | 431.0, 355.0 | 16 |
| Clobetasol propionate | 11.98 | 511.0 | 465.0, 429.4 | 10 |
| Clobetasone butyrate | 12.43 | 523.1 | 476.9, 441.3 | 7 |

[a] The underlined product ion was used for quantitative analysis.

Mobile phase composition and additives were also investigated to achieve the maximum sensitivity.

## EXPERIMENTAL

### Chemicals and reagents

Glucocorticoids (prednisone, prednisolone, cortisone, aldosterone, hydrocortisone, methylprednisolone, fluorometholone, dexamethasone, triamcinolone, beclomethasone, flumethasone, fludrocortisone acetate, budesonide, triamcinolone acetonide, fluocinolone acetonide, clobetasol propionate, clobetasone butyrate) and cortisol (9,12,12-D3), the internal standard, were purchased from Sigma (St. Louis, MO, USA). All standards were stored at −20°C. *Helix pomatia* juice was purchased from Roche Diagnostica GmbH (Mannhein, Germany) and formic acid (HCOOH, 99%) from Acros Organics (New Jersey, USA). Ultra-pure water was made using a Milli-Q Ultra pure system (Millipore, Bedford, MA, USA). The organic solvents such as methanol (MeOH), acetonitrile (ACN), and hexane were purchased from Fisher Scientific (Fair Lawn, NJ, USA). All solvents used in sample preparation and chromatographic separations were HPLC grade. Oasis HLB, Sep-pak silica and aminopropyl solid-phase extraction (SPE) cartridges containing 500 mg materials (6 mL) were purchased from Waters Co. (Milford, MA, USA). The chemical structures of all analytes are shown in Fig. 1.

### Liquid chromatography

Chromatographic separation was carried out on a ACQUITY UPLC™ system from Waters Co. using an ACQUITY UPLC™ BEH C18 column (100 mm × 2.1 mm, 1.7 μm particle size). The column oven temperature was 40°C, the flow rate was 0.3 mL/min, and the injection volume was 2 μL. The mobile phase consisted of methanol (A) and water containing 0.1% (v/v) formic acid (B). The initial composition was 35% A and 65% B. A gradient elution was performed where phase A was increased linearly to 40% in the first 6.00 min, then increased to 80% in another 6.00 min, then increased to 95% in 3.00 min, and finally returned to the initial composition in 0.10 min. The column was then equilibrated for 4 min before the next injection.

### Mass spectrometry

Mass spectrometry was carried out on a Micromass-Quattro Ultima™ Pt mass spectrometer (Waters Co.) using the negative electrospray ionization (ESI) mode. The capillary voltage was set at 3.0 kV. The cone voltage was held at 45 V. The multiplier voltage was 650 V. Nitrogen was used as the nebulizing, desolvation and cone gas. The nebulizing gas was adjusted to the maximum, and the flows of the desolvation gas and cone gas were set to 450 and 0 L/h, respectively. The source and desolvation temperatures were held at 100 and 350°C, respectively. RF lens 1 and RF lens 2 were set at 27 and 0 V, respectively. Ion energy 1 and ion energy 2 were held at 1.5 and 1.0 V, respectively. The entrance and exit slits were set at 0 and 10, respectively. During tandem mass spectrometric analysis, UHP argon was used as the collision gas, and the pressure of the collision chamber was held at $3.3 \times 10^{-3}$ mbar. The retention times, collision energies and *m*/*z* values of the precursor and product ions for each analyte are listed in Table 1. Figure 2 shows the chromatograms of all the analytes at the given elution gradient. Two peaks were obtained for triamcinolone, which may be attributed to the existence of isomers.

### Sample preparation

The sample preparation was based on our previous method[32] with some modifications. An amount of 5 g of each sample was weighed, and transferred into a 100 mL glass conical flask and spiked with 5 ng internal standard. Then 10 mL of 0.2 mol/L acetate buffer (pH 5.2) were added and the solution was sonicated for about 5 min. The pH of each mixture was adjusted to 5.2 and 100 μL β-glucuronidase/arylsulfatase from *Helix pomatia* was added. The solution was then incubated overnight at 37°C. After the mixture had been cooled to room temperature, 35 mL of methanol were added and the mixture was homogenized for 2 min. Then the





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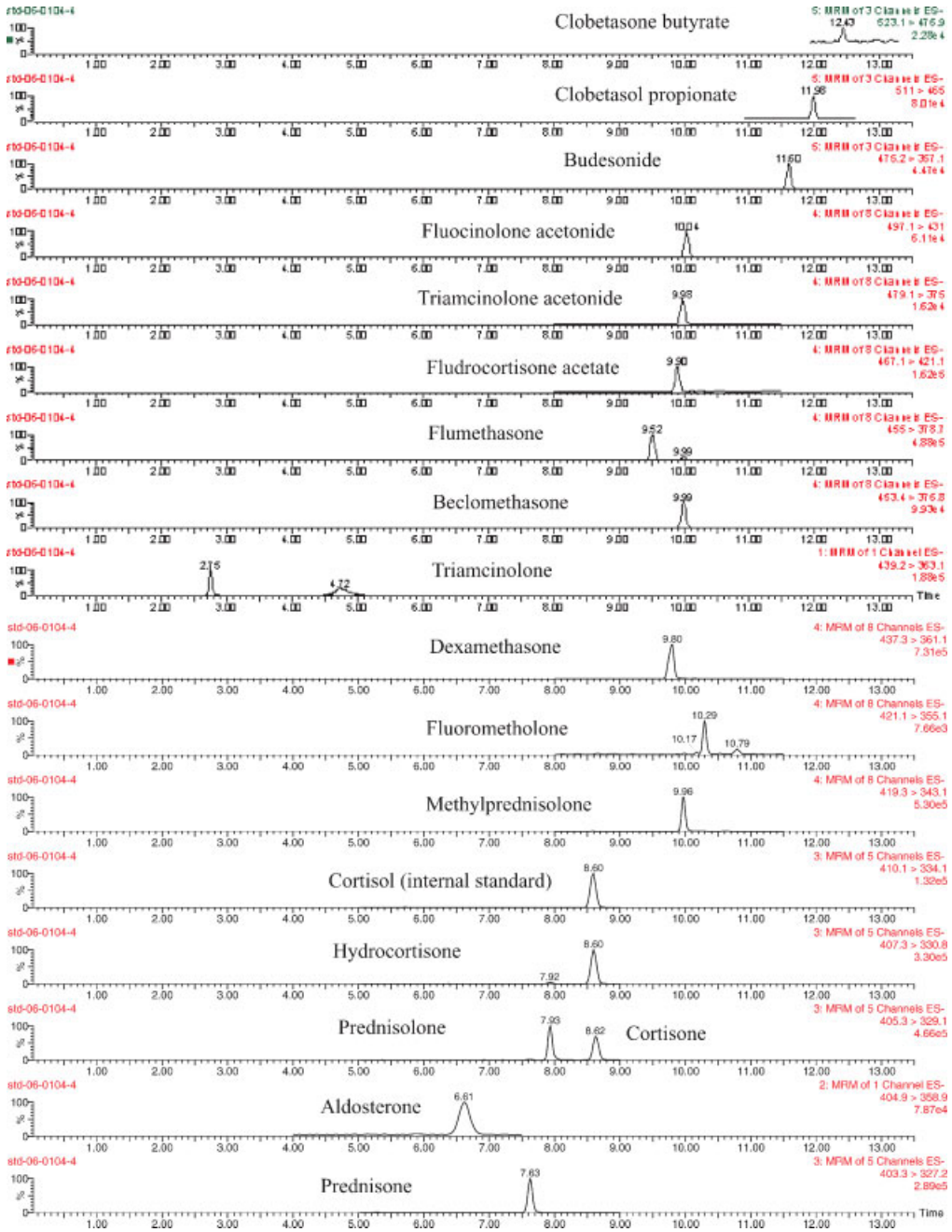


**Figure 2.** UPLC/MS/MS chromatograms of standard solution (2 μg/L) and internal standard (5 μg/L).

mixture was centrifuged at 2000 *g* for 10 min at 0°C. The supernatant was decanted into a separatory funnel and extracted twice with 20 mL *n*-hexane to remove the fat. The upper layer was discarded (*n*-hexane) and 5 mL of 1-propanol were added to prevent foaming during evaporation. The solvent was evaporated and the residue was then redissolved in 32 mL of water and 8 mL of methanol. The solution was then subjected to SPE.

An HLB cartridge was conditioned first with 6 mL of methanol and then with 6 mL of water. The solution was applied to the cartridge at a flow rate of 1–2 mL/min. The solution flask and cartridge were rinsed twice with 3 mL of water. The cartridge was dried with high-purity nitrogen. The analytes were eluted with 6 mL of methanol. The eluate was dried under a gentle nitrogen stream and then the residue was dissolved in 0.5 mL chloroform, and 5 mL of *n*-hexane by ultrasonication for 30 s. The solution then was passed through a Sep-Pak silica SPE cartridge conditioned with 6 mL of *n*-hexane without any pressure. The cartridge was washed with 5 mL of *n*-hexane to remove sample interferences. The analytes were then eluted with 6 mL of ethyl acetate saturated water. The eluate was dried under a gentle nitrogen stream, and the residue was eluted with 2 mL methanol/ethyl acetate (40:60, v/v) by an aminopropyl SPE cartridge conditioned with 4 mL methanol/ethyl acetate (40:60, v/v) and 4 mL ethyl acetate saturated water. The





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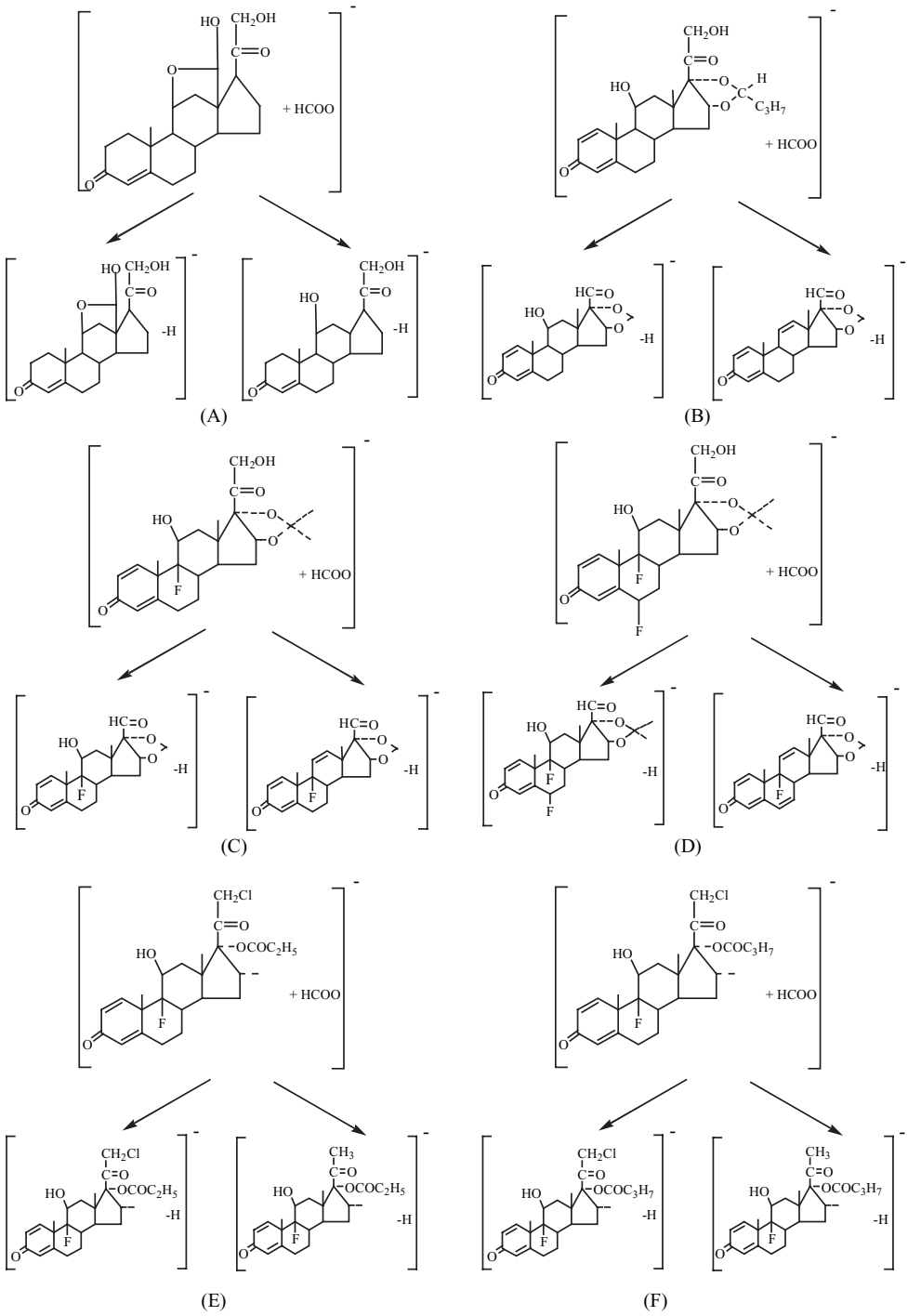


**Figure 3.** Proposed fragmentation scheme for collision-induced dissociation of aldosterone (A), budesonide (B), triamcinolone acetonide (C), fluocinolone acetonide (D), clobetasol propionate (E), and clobetasone butyrate (F).

eluate was dried under a gentle nitrogen stream. The residue was reconstituted with 1 mL methanol and mixed with a vortex stirrer.

## Method validation and calculations

Stock solutions containing all 17 glucocorticoids were prepared at different concentrations by dissolving each pure standard in methanol. Each stock solution had the same concentration of each of the glucocorticoids. These solutions were stored in the dark at −20°C and used over the course of 6 months. All calculations were based on the peak area ratio relative to that of the internal standard (IS). The concentration of the IS in all the calibration mixtures and in the final sample solutions was 5 μg/L.

Calibration samples were prepared at six different concentrations in the range of 1–200 μg/L, by diluting appropriate amounts of the stock solutions with egg and milk matrices. Calibration curves were obtained by plotting the





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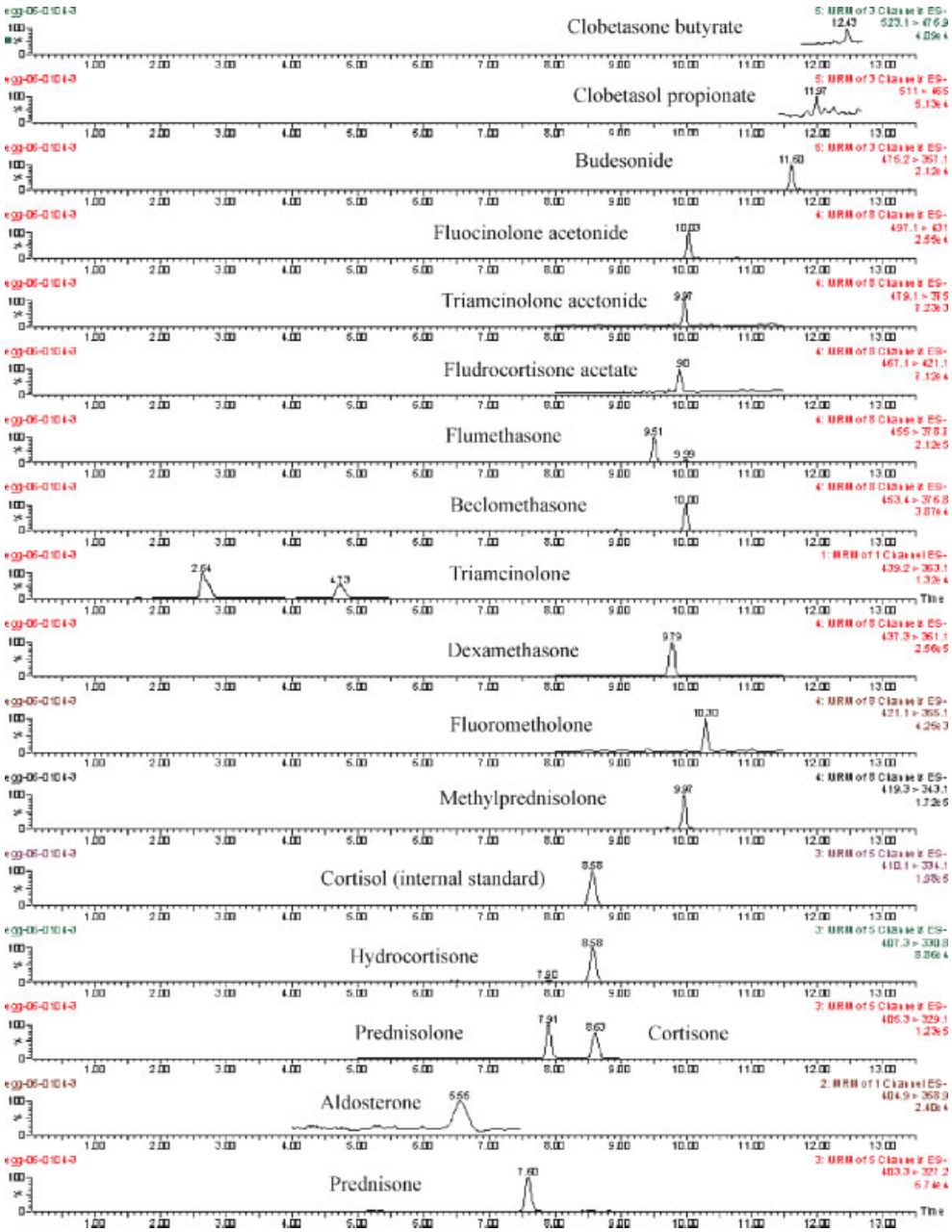


**Figure 4.** Chromatogram of spiked egg sample containing 0.40 μg/kg of each compound and 1.00 μg/kg internal standard.

ratios of the analyte peaks and the IS peak areas against the analyte concentrations, with the results analyzed by linear regression. The recovery for each glucocorticoid was determined by comparing the response of the test samples and the calibration samples at two different concentration levels. The test samples were obtained from spiked egg and milk samples and subjected to SPE with subsequent UPLC/MS/MS analysis according to the procedures described above. Each glucocorticoid recovery was assessed by comparing the peak area ratios for six replicates of an extracted sample to the calibration counterparts representing 100% recovery. The precision expressed as percent relative standard deviation (% RSD) was determined for each glucocorticoid from six replicates of spiked egg and milk samples. The limit of detection (LOD) and the limit of quantification (LOQ) for each glucocorticoid are defined as the minimum concentrations needed to produce a signal greater than 3 and 10 times the signal-to-noise, respectively. For each analyte, the within- and the between-day reproducibilities were determined by testing six replicates of independently extracted samples for each control concentration on five different days.

## RESULTS AND DISCUSSION

### UPLC/ESI-MS/MS

Previous chromatography/ESI-MS studies of glucocorticoid residues have reported different forms for the precursor





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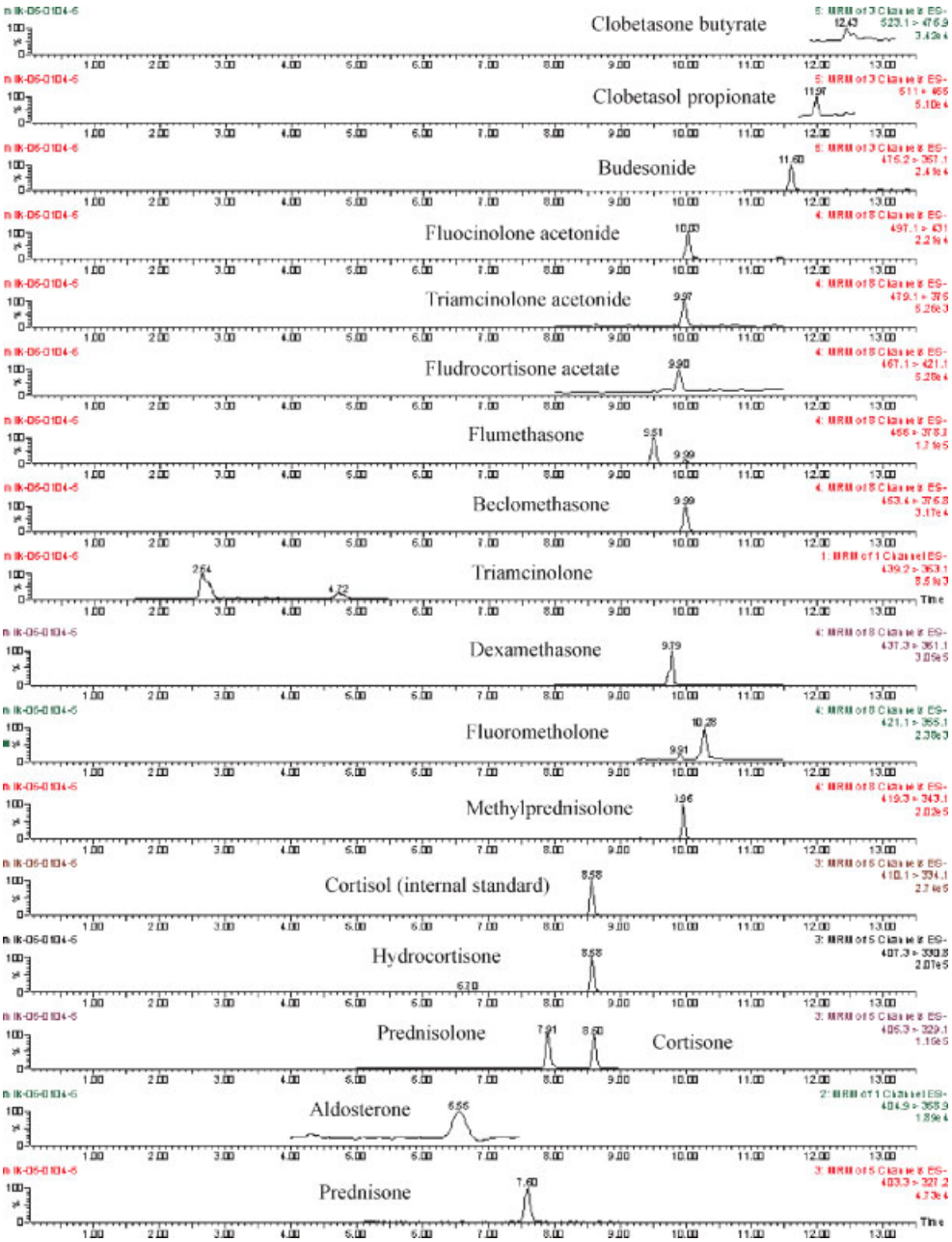


**Figure 5.** Chromatogram of spiked milk sample containing 0.40 μg/kg of each compound and 1.00 μg/kg internal standard.

ions.[21–31] Fiori *et al*.[21] reported $[M+H]^+$ as the precursor ion using a methanol/water (1% acetic acid) mobile phase. Brambilla *et al*.[25] determined $[M-H]^-$ to be the precursor ion with a methanol/ammonium/formate mobile phase, and Van den Hauwe *et al*.[28] documented $[M+HCOO]^-$ as the precursor ion with acetonitrile/water (90/10, v/v) + 0.3% formic acid as the mobile phase. It is clear that the precursor ion depends on the additive in the mobile phase. In this study, with formic acid used as the additive, $[M+formate]^-$ was the most abundant peak in the mass spectra and it was identified as the precursor ion.

Concentrations of formic acid additive from 0.05% to 0.30% (v/v) were evaluated. The results indicate that the addition of formic acid improves the signal intensity. When formic acid is less than 0.1% (v/v), the formation of $[M+formate]^-$ adducts is difficult. On the other hand, higher formic acid concentrations can cause ion-masking and suppress the ionization efficiency. A significantly higher response was obtained with a methanol/water mobile phase than with an acetonitrile/water mobile phase, which is different from previously reported results.[28] Therefore, a mixture of methanol and water containing 0.1% (v/v) formic acid was selected as mobile phase. After the precursor ions had been determined, the product ion scan mode was used to identify the product ions for 17 glucocorticoids. Figure 3 shows the proposed fragmentation scheme for the collision-induced dissociation (CID) of aldosterone, budesonide, triamcinolone acetonide, fluocinolone acetonide, clobetasol





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**Table 2.** Linearity for each component (y: peak area ratio of the compound and internal standard; x: mass concentration of the compound, μg/L)

| Compound | Calibration equation[a] | r[a] | Calibration equation[b] | r[b] |
|---|---|---|---|---|
| Prednisone | $y = 0.1262x - 0.1166$ | 0.9984 | $y = 0.1151x - 0.0247$ | 0.9999 |
| Prednisolone | $y = 0.2839x - 0.1286$ | 0.9999 | $y = 0.2859x + 0.1569$ | 0.9959 |
| Cortisone | $y = 0.2847x - 0.1539$ | 0.9998 | $y = 0.2872x + 0.0875$ | 0.9969 |
| Aldosterone | $y = 0.0563x + 0.0765$ | 0.9972 | $y = 0.0663x + 0.0403$ | 0.9918 |
| Hydrocortisone | $y = 0.2760x - 0.0631$ | 0.9999 | $y = 0.2644x + 0.5374$ | 0.9947 |
| Methylprednisolone | $y = 0.2941x + 0.2273$ | 0.9994 | $y = 0.2998x - 0.3125$ | 0.9983 |
| Fluorometholone | $y = 0.0046x + 0.0009$ | 0.9987 | $y = 0.0048x + 0.0022$ | 0.9953 |
| Dexamethasone | $y = 0.7800x - 1.1548$ | 0.9974 | $y = 0.7319x - 0.3273$ | 0.9999 |
| Triamcinolone | $y = 0.0660x - 0.1207$ | 0.9982 | $y = 0.0635x - 0.0290$ | 0.9973 |
| Beclomethasone | $y = 0.0962x - 0.0593$ | 0.9997 | $y = 0.0965x - 0.1333$ | 0.9980 |
| Flumethasone | $y = 0.3512x - 0.2084$ | 0.9999 | $y = 0.3526x + 0.2301$ | 0.9935 |
| Fludrocortisone acetate | $y = 0.1366x - 0.1291$ | 0.9989 | $y = 0.1406x + 0.0440$ | 0.9973 |
| Budesonide | $y = 0.0134x - 0.0002$ | 0.9998 | $y = 0.0136x + 0.0213$ | 0.9938 |
| Triamcinolone acetonide | $y = 0.0382x - 0.0120$ | 0.9999 | $y = 0.0417x + 0.0340$ | 0.9998 |
| Fluocinolone acetonide | $y = 0.0657x - 0.0669$ | 0.9989 | $y = 0.0671x - 0.0025$ | 0.9988 |
| Clobetasol propionate | $y = 0.0640x - 0.0112$ | 0.9999 | $y = 0.0625x - 0.0042$ | 0.9977 |
| Clobetasone butyrate | $y = 0.0047x + 0.0071$ | 0.9906 | $y = 0.0067x - 0.0070$ | 0.9918 |

[a] Egg matrix; [b] milk matrix.

propionate and clobetasone butyrate. By loss of formic acid and water from the precursor ion, $[M–H]^-$ and $[M–H–H_2O]^-$ are the major fragmentation ions for fludrocortisone acetate. The major fragmentation ions for the other glucocorticoids are $[M–H]^-$ and $[M–H–CH_2O]^-$, which are due to the loss of formic acid and then the loss of formaldehyde from the hydroxymethyl group (C21).

## Optimization of sample preparation

Because of the low solubility of the glucocorticoids in water, methanol was used to extract these compounds. In order to determine ultra-trace levels of the target analytes, it is necessary to eliminate possible interferences from the samples. The previously established clean-up method, described in the Experimental section, was used. Figures 4 and 5 present the chromatograms of 17 glucocorticoids in eggs and milk spiked at 0.40 μg/kg. The chromatograms show that even at this low level, all the target compounds are determined without interference from the biological matrix.

## Validation of the overall procedure

Validation experiments employing calibration samples made up with egg and milk matrices were performed to evaluate the linearity of the method. Measurements for each glucocorticoid in milk and eggs were linear over a wide range (1–200 μg/L) with good correlation coefficients (>0.99), as shown in Table 2. The recoveries were evaluated by spiking two 5 g samples (milk or eggs) with either 2 or 10 ng of each standard analyte and 5 ng of IS and then analyzing each sample in replicates of six. The results are listed in Tables 3 and 4. The average recoveries of each compound ranged from 61.5% to 119.6%. Chromatograms of

**Table 3.** Spiked recoveries, relative standard deviations (RSD), LOD and LOQ of spiked eggs (n = 6)

| | Spiked level (0.40 μg/kg) | | Spiked level (2.00 μg/kg) | | | |
|---|---|---|---|---|---|---|
| Compound | Recovery% | RSD% | Recovery% | RSD% | LOD (μg/kg) | LOQ (μg/kg) |
| Prednisone | 92.5 | 3.5 | 91.4 | 6.1 | 0.02 | 0.06 |
| Prednisolone | 98.3 | 3.4 | 99.6 | 11.2 | 0.05 | 0.15 |
| Cortisone | 65.6 | 5.2 | 72.6 | 10.1 | 0.02 | 0.08 |
| Aldosterone | 93.8 | 3.7 | 102.5 | 5.9 | 0.01 | 0.04 |
| Hydrocortisone | 92.0 | 5.6 | 100.4 | 3.4 | 0.02 | 0.07 |
| Methylprednisolone | 89.6 | 3.2 | 90.8 | 6.4 | 0.04 | 0.14 |
| Fluorometholone | 86.5 | 5.3 | 89.6 | 7.6 | 0.38 | 1.27 |
| Dexamethasone | 99.3 | 4.3 | 112.3 | 7.1 | 0.01 | 0.04 |
| Triamcinolone | 69.5 | 13.9 | 78.9 | 17.0 | 0.12 | 0.42 |
| Beclomethasone | 105.7 | 6.9 | 111.4 | 12.0 | 0.06 | 0.19 |
| Flumethasone | 81.6 | 8.4 | 89.5 | 3.5 | 0.01 | 0.04 |
| Fludrocortisone acetate | 89.4 | 8.9 | 95.1 | 12.7 | 0.09 | 0.29 |
| Budesonide | 98.5 | 10.4 | 106.7 | 7.1 | 0.04 | 0.14 |
| Triamcinolone acetonide | 95.1 | 4.8 | 108.3 | 11.4 | 0.03 | 0.08 |
| Fluocinolone acetonide | 112.5 | 3.5 | 111.6 | 11.0 | 0.02 | 0.08 |
| Clobetasol propionate | 98.3 | 1.8 | 106.4 | 4.6 | 0.33 | 1.12 |
| Clobetasone butyrate | 112.9 | 4.8 | 118.7 | 6.0 | 0.37 | 1.23 |





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**Table 4.** Spiked recoveries, relative standard deviations (RSD), LOD and LOQ of spiked milk (n = 6)

| | Spiked level (0.40 μg/kg) | | Spiked level (2.00 μg/kg) | | | |
|---|---|---|---|---|---|---|
| Compound | Recovery% | RSD% | Recovery% | RSD% | LOD (μg/kg) | LOQ (μg/kg) |
| Prednisone | 96.4 | 8.9 | 98.6 | 8.9 | 0.02 | 0.06 |
| Prednisolone | 114.9 | 8.0 | 110.7 | 9.3 | 0.06 | 0.21 |
| Cortisone | 61.5 | 4.8 | 67.6 | 13.9 | 0.01 | 0.04 |
| Aldosterone | 92.9 | 2.4 | 98.6 | 10.2 | 0.01 | 0.03 |
| Hydrocortisone | 118.6 | 13.6 | 116.1 | 7.6 | 0.02 | 0.08 |
| Methylprednisolone | 101.3 | 8.3 | 118.8 | 5.4 | 0.03 | 0.09 |
| Fluorometholone | 100.1 | 16.2 | 92.8 | 13.6 | 0.22 | 0.73 |
| Dexamethasone | 97.3 | 8.5 | 95.6 | 5.7 | 0.01 | 0.03 |
| Triamcinolone | 72.5 | 3.9 | 70.3 | 7.8 | 0.08 | 0.28 |
| Beclomethasone | 86.4 | 11.8 | 98.6 | 5.9 | 0.04 | 0.15 |
| Flumethasone | 93.1 | 8.1 | 108.5 | 6.4 | 0.01 | 0.03 |
| Fludrocortisone acetate | 87.2 | 4.9 | 89.6 | 16.3 | 0.07 | 0.23 |
| Budesonide | 107.3 | 16.7 | 108.5 | 9.7 | 0.03 | 0.09 |
| Triamcinolone acetonide | 119.6 | 10.3 | 112.8 | 6.8 | 0.01 | 0.05 |
| Fluocinolone acetonide | 100.3 | 3.5 | 114.6 | 5.5 | 0.02 | 0.05 |
| Clobetasol propionate | 103.1 | 3.6 | 95.8 | 16.7 | 0.13 | 0.43 |
| Clobetasone butyrate | 115.2 | 18.1 | 86.9 | 18.4 | 0.17 | 0.57 |

spiked egg and milk samples containing 0.40 μg/kg of each compound and 1.00 μg/kg of IS are shown in Figs. 4 and 5, respectively. For each compound, the precision of this method, represented by RSD, at each fortification level is summarized in Tables 3 and 4. The results show that the precision of the method is within 20%, which is very satisfactory. For each analyte, the within- and between-day reproducibilities were determined by testing six replicates of independently extracted samples for each control concentration on five different days. Two control concentrations (2 and 10 μg/L) were used. The within-day reproducibility ranged from 3.6% to 8.6% and the between-day reproducibility ranged from 4.8% to 11.5%. The LODs ranged from 0.01 to 0.38 μg/kg for eggs (Table 3) and from 0.01 to 0.22 μg/kg for milk (Table 4). The LOQs ranged from 0.04 to 1.27 μg/kg for eggs (Table 3) and from 0.03 to 0.73 μg/kg for milk (Table 4). Compared with previously published methods, the LOD of this method is approximately the same or less.

Five milk samples commercially available from the local market were analyzed for the 17 glucocorticoids using the above method. Figure 6 shows the ion chromatograms of a milk sample; two peaks were found to be the same retention times as prednisolone and hydrocortisone. However, in terms of EU analytical criteria, the ion chromatogram area ratios 405.3 > 329.1/405.3 > 359.0 and 407.3 > 330.8/407.3 > 361.1 in the milk sample are obviously different from the standard sample. Therefore, the two peaks found in the milk sample are confirmed not to be prednisolone and

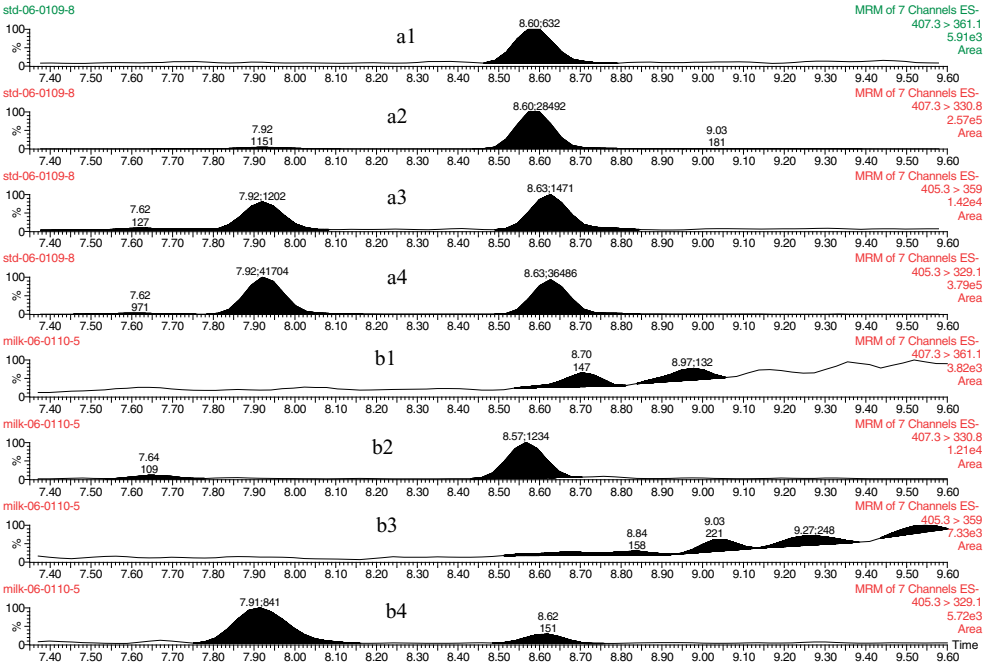


**Figure 6.** Chromatogram of hydrocortisone, prednisolone of standard sample (a1–a4) and blank milk sample (b1–b4).





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hydrocortisone, and in fact none of the glucocorticoids were found in the commercial milk samples. Five egg samples from the local market were also tested for the 17 glucocorticoids and no positive samples were found.

## CONCLUSIONS

In this investigation, a comprehensive analytical method was developed for simultaneous extraction and determination of 17 glucocorticoids in eggs and milk. The method demonstrates good efficiency, linearity, accuracy and precision. Good recoveries ranging from 61.5% to 119.6% were obtained. The LOD of this method was approximately the same or less than previously reported GC/MS and LC/MS methods. This new method may be suitable for the surveillance of the abuse of glucocorticoid compounds in eggs and milk.

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