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Elucidation of two new corticosteroids, betamethasone dibutyrate and betamethasone tributyrate

Shin-Yu Chen [a], Mingchih Fang [b,*], Ya-Tze Lin [a], Chia-Fen Tsai [c], Hwei-Fang Cheng [a], Der-Yuan Wang [a]

[a] Division of Research and Analysis, Taiwan Food and Drug Administration, Taipei, Taiwan
[b] Department of Food Science, National Taiwan Ocean University, Keelung, Taiwan
[c] Southern Center for Regional Administration, Taiwan Food and Drug Administration, Kaohsiung, Taiwan

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ABSTRACT

Corticosteroids were used normally as anti-inflammatory drugs. However, in some area certain corticosteroids might be illegally used as growth promoting agent in feed, and as prohibited doping substances in game and sport for human or/and animal performance-enhancing. Synthesized structural similar corticosteroids were popular in black market because they can pass routine drug screening. In this study two new artificial synthesized corticosteroids were found in claimed hydrolyzed wheat product. Liquid chromatography coupled with high resolution mass spectrometry (HRMS, Orbitrap) was applied to separate and elucidate the corticosteroids in the sample. Two unknown peaks with optical spectra similar to corticosteroids were first screened out at the beginning, and then their accurate molecular weight ($M + H^+$) m/z 533.29059 and m/z 603.33289 were detected by HRMS. Element formulas of unknowns were calculated by the accurate mass and isotopes abundance. Structures were proposed by their fragment ions at high energy collision dissociation (HCD, 10 eV) and compared with candidate standard compounds. The two unknowns shared similar molecular skeleton with steroid core structure and presented man made fluorine element in their molecule. As the results, the unknowns in the sample were artificial synthesized, and the sample product was not a real food. The detected corticosteroids were also synthesized as reference compounds for conformation. Two new corticosteroids named betamethasone dibutyrate and betamethasone tributyrate were found and first time reported in this work. The legality of structural similar/modified corticosteroids were blurry and their safety were unverified. The confirmed identifications of two new found corticosteroids, and their mass spectra were provided in this paper for the reference of drug detection.

1. Introduction

Corticosteroids are known drugs for the treatment of inflammatory diseases such as arthritis, asthma, and rhinitis. They temporarily relieve the inflammatory symptoms, and should be controlled for medical use. The illegal uses of corticosteroids for anti-inflammatory function have been reported in cosmetics, natural products, and counterfeit drug [5,7]. Corticosteroids also are introduced in animal production as growth promoting agent, and often added illegally into milk replacers of feed [6]. Other illegal uses are applied as performance-enhancing drug (doping) for athlete due to it can cause euphoria and alleviate pain in general [2]. Despite corticosteroids are banned in non-medical product, the counterfeit products might be present on the market, and cause health risks.

The detections of corticosteroids in various samples such as water, foods, feeds, environmental samples, pharmaceuticals, human body samples (plasma, urine), and cosmetics were mainly achieved by HPLC coupled with mass spectrometry, which offered good sensitivity and selectivity for simultaneous quantification of multiple corticosteroids from complex matrix [8,9]. In our case, a food product (claimed hydrolyzed wheat solution) packaged in an injection bottle was investigated. Due to the weird packaging style (injection bottle) for a food product, it was recognized and linked to adulterated product and drug abuse. Therefore, sample was sent to commercial testing laboratory for screening of drug active ingredients including 13 corticosteriods (betamethason, betamethason dipropinate, betamethason valerate,

* Corresponding author at: No. 2 Beining Rd., Keelung City 20224, Taiwan.
E-mail address: mcfang@mail.ntou.edu.tw (M. Fang).

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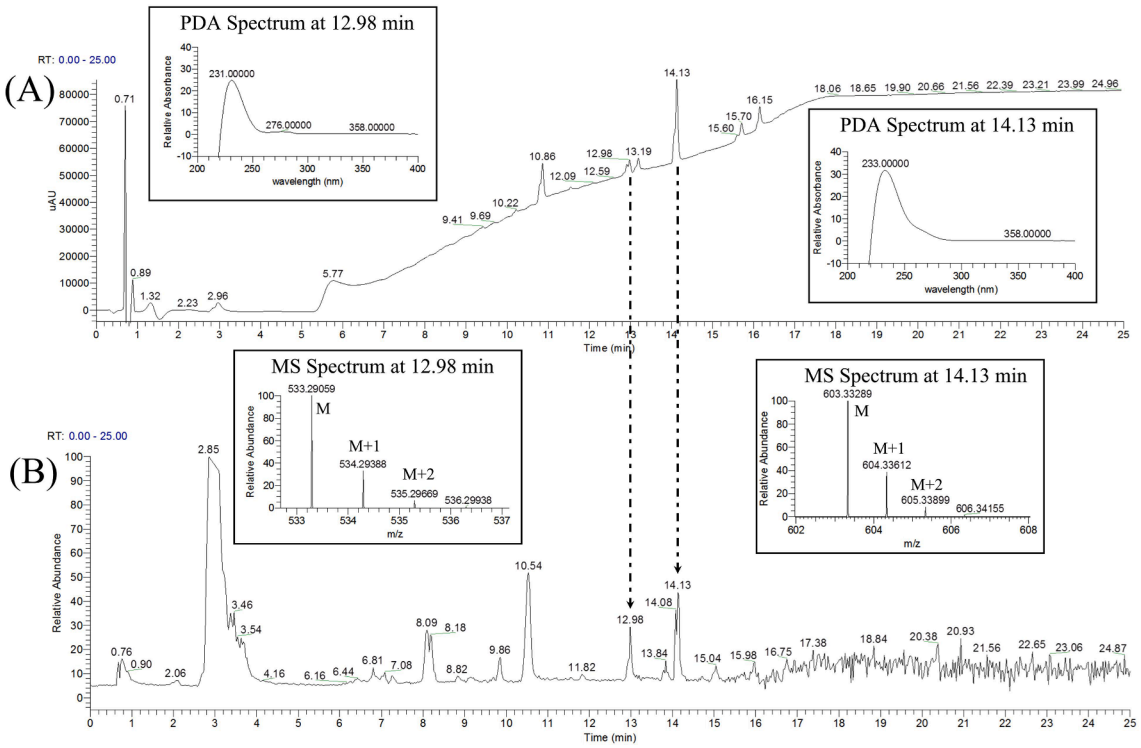


Fig. 1. Sample chromatogram monitored at 230 nm (A) and total ion chromatogram (TIC) (B). The PDA and MS spectra of peaks at RT = 12.98 and 14.13 min were point out in small figures. Data was acquired by linked PDA and MS.

beclomethasone dipropionate, clobetasol propionate, cortison acetate, dexamethasone, fluocinonide, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone acetonide), 10 estrogens (estradiol, estradiol benzoate, estriol, estrone, ethinyl estradiol, ethisterone, flibanserin, norethisterone acetate, norgestrel, progesterone), and 17 antibiotics (ampicillin, clindamycin, clindamycin phosphate, cephalexin, cephradine, cefazolin, ceftriaxone, ciprofloxacin, chloramphenicol, doxycycline, erythromycin, lincomycin, minocyclin, rifampin, tetracycline, gentamicin, neomycin). Along with onabotulinum toxin A, there were total 41 drug substances screened. However, none of them was detected. The sample was introduced into LC coupled with high resolution mass spectrometer and photodiode array detector. Two new corticosteroids were found and reference compounds were synthesized for confirmation. The structure elucidation and the mass spectra of the new corticosteroids were reported in this paper.

2. Materials and methods

Imported food product in injection bottle was confiscated by customs. Sample was provided with incomplete packaging. The bottle was not labeled, and came with a food product brochure. Liquid 0.5 mL was taken by a syringe from the injection bottle, diluted with methanol to 5 mL before analysis. Ethylmagnesium bromide solution (3.0 M in diethyl ether), betamethasone, dexamethasone, and butyryl chloride were form Sigma-Aldrich (St. Louis, Mo, USA).

2.1. Separation and detection

LC system (UltiMate 3000, Thermo Fisher Scientific Inc. Waltham, MA, USA), an Poroshell 120 C18 column (2.1 × 100 mm, 1.7 μm particle size, Agilent Inc, Santa Clara, CA, USA), and the eluent consisted of 5 mM ammonia acetate water solution (A) and acetonitrile with 5 mM ammonia acetate (B) was utilized. The linear gradient elution for sample was programed as: 0–2 min, 2% B; 2–14 min, 2–100% B; 14–19 min, 100% B; 19–20 min, 100–2% B; 20–25 min, 2% B. The flow rate was set at 500 μL/min. For synthesized reference compounds and sample reconfirmation, the linear gradient elution was programed as: 0–2 min, 40% B; 2–14 min, 40–100% B; 14–19 min, 100% B; 19–20 min, 100–40% B; 20–25 min, 40% B. Mass spectrometer was a Q Exactive Plus Hybrid Quadruple-Orbitrap Mass Spectrometer (Thermo Fisher Scientific Inc.). The heater and capillary temperature was set to 250 °C and 240 °C, respectively. The capillary of the electrospray ionization (ESI) interface was set to 3.8 kV. Sheath gas and auxiliary gas were set to 50 and 5 units, respectively. Scan range was *m/z* 100∼1000 with resolution 140 k (defined at *m/z* = 200 and was set at full width at half maximum). C-trap allowed 1×10^6 charges, and the maximum injection time was set 100 ms. Procurer ions with window 1 amu were fragmented by high energy collision dissociation (HCD, set 10 eV) to obtain MS/MS spectra. Accurate molecular weight, fragments, and isotopic abundance were used for compound prediction. The system contained a photodiode array detector (PDA, DAD-3000RS, Thermo Fisher Scientific Inc.) between LC column and Q-Orbitrap MS, monitored at 200 nm to 400 nm.

Table 1
The possible elemental composition of unknown corticosteroid *m/z* 533 and 603 ($M + H^+$), delta mmu was limited in 1.0.

No	Formula of *m/z* 533	RDB[1]	Delta mmu[2]
1	$C_{30}H_{41}ON_2F_3P$	10.5	0.2785
2	$C_{30}H_{42}O_7F$	9.5	−0.3183
3	$C_{30}H_{48}ONPS_2$	8.0	−0.3545
4	$C_{30}H_{47}NFP_3$	9.0	0.6003
5	$C_{30}H_{40}O_2N_5P$	14.0	−0.8235
No	Formula of *m/z* 603	RDB[1]	Delta mmu[2]
1	$C_{34}H_{54}O_2NPS_2$	9.0	0.0807
2	$C_{34}H_{48}O_8F$	10.5	0.1170
3	$C_{34}H_{46}O_3N_5P$	15.0	−0.3883
4	$C_{34}H_{47}O_2N_2F_3P$	11.5	0.7137

[1] RDB, ring double bond equivalents.
[2] mmu, minimass unit.

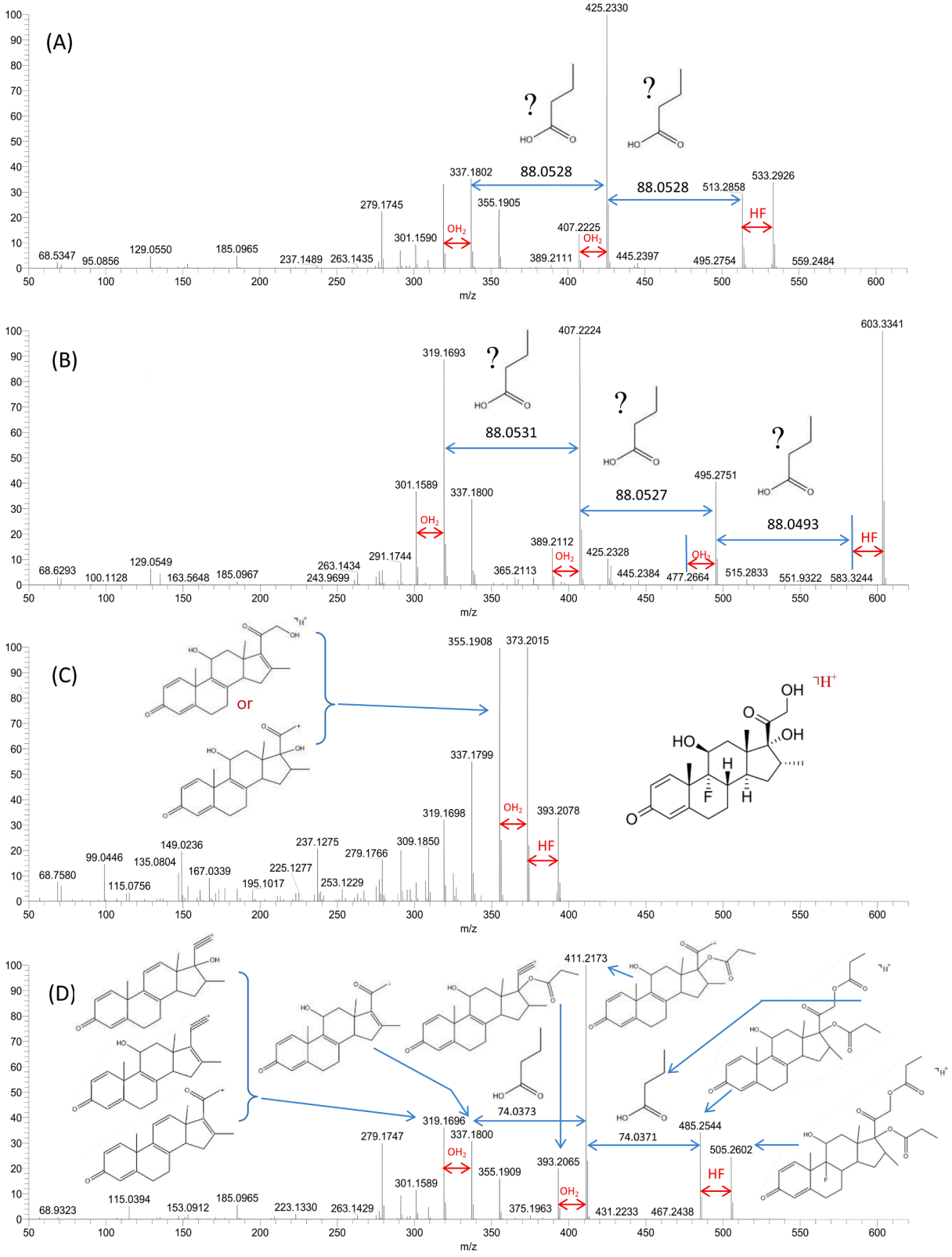


Fig. 2. Fragments of unknown *m/z* 533 (A), unknown *m/z* 603 (B), dexamethasone (C), and betamethasone dipropionate (D).

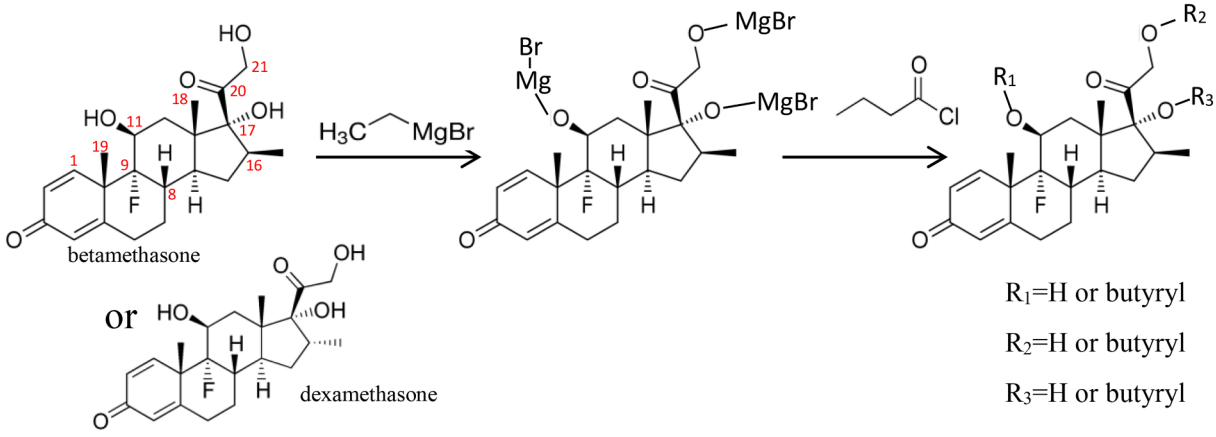


Fig. 3. Reaction scheme for the synthesis of dexa/beta-methasone mono/di/tri-butyrate.

2.2. Synthesis of reference compounds, dexamethasone dibutyrate, dexamethasone tributyrate, betamethasone dibutyrate, and betamethasone tributyrate

The synthesis of dexamethasone derivatives and betamethasone derivatives were based on the esterification reaction through Grignard reaction started from dexamethasone and betamethasone, respectively, with butyryl chloride [3]. The modified process was described as below. A three-neck round bottom flask, drying tube (filled with $CaCl_2$), condenser, and stoppers were pre dried in a 200 °C oven for at least 2 h and cooled. Apparatus were assembled for Grignard reaction. Place 0.4 g betamethasone (1 mmol) and 50 mL tetrahydrofuran in the three-neck round bottom flask with gentle nitrogen purge and stirring. Ethyl magnesium bromide 1.1 mL (3.3 mmol) was added through a syringe in 10 min. The solution was stirred for a day in room temperature. Butyryl chloride 0.48 mL (4.5 mmol) was diluted with diethyl ether (1:1, v/v, keep from air moisture) and added through a syringe into the system in 30 min. The reaction mixture was refluxed for 5 h, and then water 50 mL was added. The organic layer was separated by a separating funnel. The residual water layer was extracted with diethyl ether. The combined organic layers was washed with saturated sodium hydrogen carbonate solution, and then washed with water several times until neutral. The solution was dried over anhydrous sodium sulfate, and evaporated to obtain betamethasone derivatives. The synthesis of dexamethasone derivatives was by the similar processes to betamethasone derivatives, starting from dexamethasone instead.

3. Results and discussion

3.1. Molecular formula prediction of unknowns

The diluted sample was injected into LC coupled with PDA and HRMS detectors, chromatograms were in Fig. 1. The obtained PDA chromatogram contained only a few peaks, indicating the sample composition was relatively simple. Therefore, the sample might not be a natural food product as it claimed, and more like to be an artificial formulation. The absorption spectra of every peak were checked, two peaks at retention time (RT) 12.98 and 14.13 were pointed out due to their similar spectra (Fig. 1A) to corticosteroids which had a characteristic value of maximum absorbance at near 230 nm. Prior to this study, the sample was sent to a commercial testing laboratory for 41 drugs screening including 13 corticosteroids, but none of them was detected. In other words, there was no target matched the detection list. The detailed HRMS spectra of the two suspicious unknown corticosteroids were obtained from the MS chromatogram (Fig. 1B) on the same retention time to PDA chromatogram. The total tubing volume was about 15 μL between the PDA and HRMS detectors in LC system which was estimated to contribute 0.03 min delay time between PDA and MS peaks detection. Normally the peaks in chromatograms of PDA and MS were not all matched because signal was strongly influenced by compound structure, functional group, and ionization efficiency. In Fig. 1 B, two peaks with similar RT to PDA detection can be found. The MS spectra of these two suspicious peaks were shown in Fig. 1B. Two corticosteroids with accurate molecular weight ($M + H^+$) *m/z* 533.29059 and *m/z* 603.33289 were tentatively detected.

The abundance of M + 1 isotope for compound *m/z* 533 was 31.5% to its monoisotope which was set to 100% (Fig. 1B). The numbers of carbon in the compound can be calculated by the formula: possibility of $^{13}C = C_1^n \times 0.011 \times 0.989^{(n-1)} = 31.5/131.5$, where 0.011 and 0.989 was the natural abundance of ^{13}C and ^{12}C, respectively. The 31.5 divided by 131.5 was the experimental value of ^{13}C portion to total carbon [4]. Apparently, *n* equal to 30, the unknown compound of *m/z* 533 contained 30 carbons. The relative abundance of M + 2 was low, indicated there was no halogen (Cl or Br) except fluorine (natural abundance of ^{19}F: 100%; ^{35}Cl:76%, ^{37}Cl:24%; ^{79}Br:51%, ^{81}Br:49%). The same method was applied into the other compound of *m/z* 603. The possible element compositions of unknowns *m/z* 533 and 603 were calculated by Xcalibur softwave (Thermo Fisher Scientific Inc.) using parameters of accurate mass 533.29059 and 603.33289 ($M + H^+$, delta mmu = 1), carbon = 30 and 34, respectively. Principal elements of organic compound such as C, N, O, H, F, S, P, and Na were in used. The possible element compositions were conjectured as Table 1. The procurer ions of *m/z* 533 and 603 were selected (1 unit isolation window) and fragmented at HCD = 10 eV by mass spectrometer, the spectra of fragment ions were in Fig. 2A and B. Three characteristic ions *m/z* 319, 337, and 355 [12] grabbed great attention of us because they were similar to methasone core steroid. The spectrum of methasone (Fig. 2C) was compared to the sample, and showed various similarities. The loss of fragment HF in Fig. 2A (533.2926–513.28558 = 20.0068, matched theoretical value of HF = 1.0078 + 18.9984 = 20.0062) indicated that the unknown corticosteroid contained fluorine in its molecule which similar to the spectrum of standard compound, dexamethasone (Fig. 2C). Dexamethasone was an artificial corticosteroid, the man-made fluorine in the molecule made the compound more stable than its natural counterpart which had a hydrogen instead of fluorine on its molecule. As the results, the unknown corticosteroids in the sample were artificial synthesized, and the sample product was not a real food as it claimed. It was necessary to identify the two unknown corticosteroids.

European pharmacopoeia and US pharmacopoeia recorded several methasone relative drugs and their structure similar impurities. None of them was reported as mass equal to 532 or 602 amu. The biggest methasone derivative available in commercial was betamethasone butyrate propionate with molecular mass of 518.6 g/mol. Due to the availability of methasone derivative compound in our laboratory,

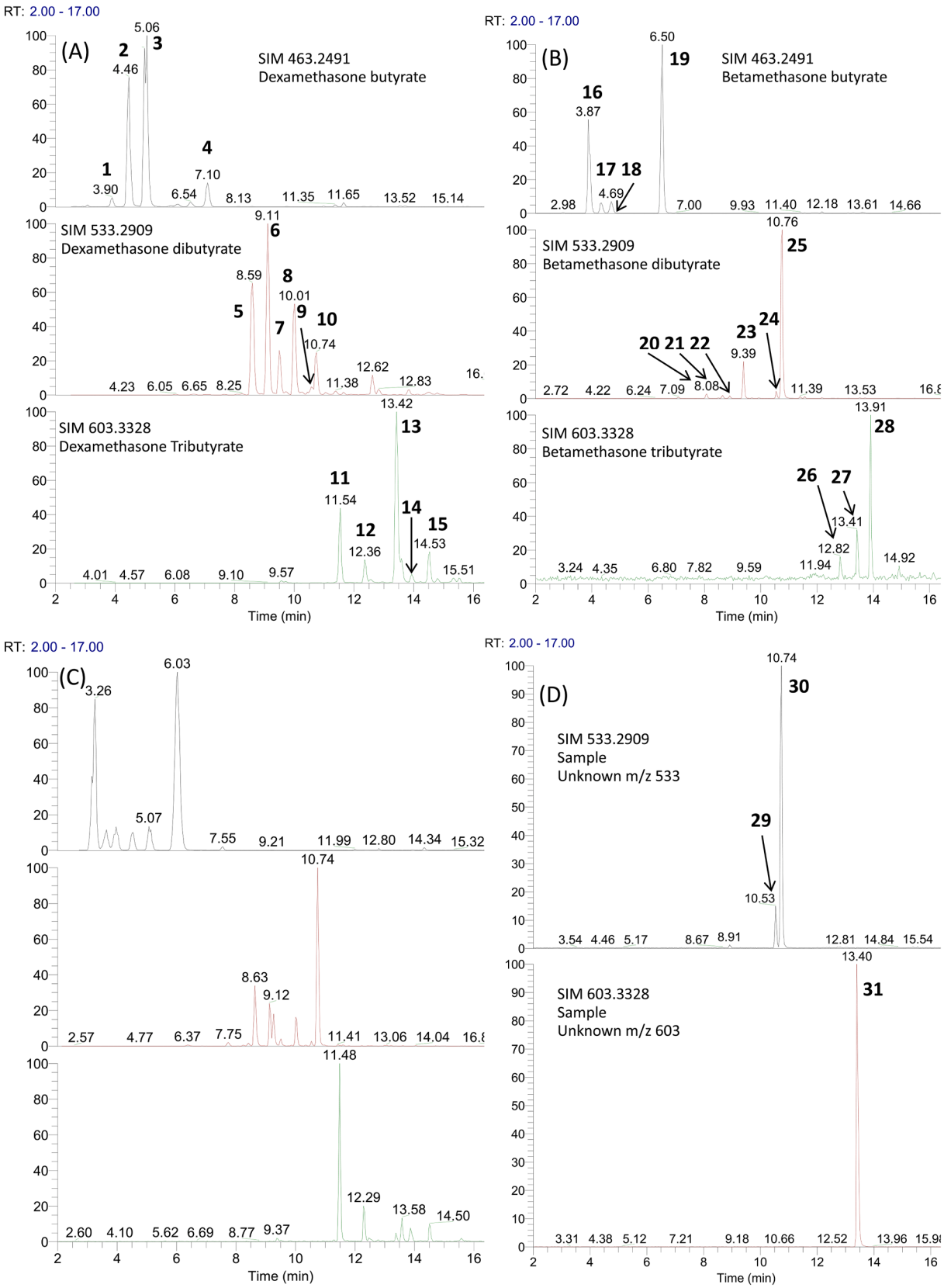


Fig. 4. Chromatograms of synthesized methasone derivatives and sample. Dexamethasone derivatives (A); Betamethasone derivatives (B); Dexamethasone derivatives after storage (C); Sample (D).

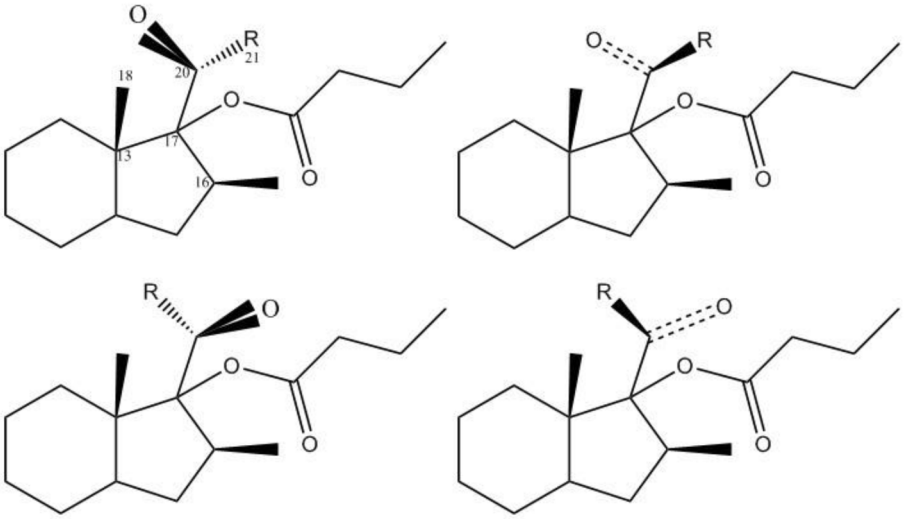
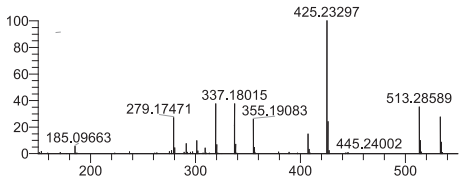
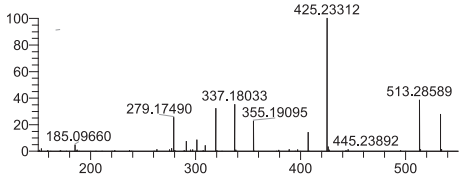
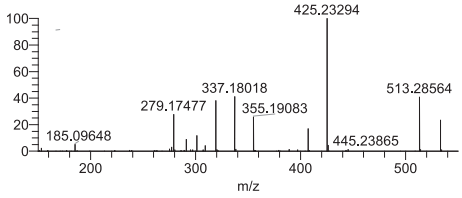


Fig. 5. Rotational transformations of derived ketol side chain existing four preferred conformations.

Table 2
MS spectra of dexa/beta-methasone dibutyrate and unknown *m/z* 533 in sample.

No	Name	Spectra, Y = relative abundance and X = *m/z*
1	Dexamethasone dibutyrate (peak 10)	
2		
	Betamethasone dibutyrate (peak 25)	
3		
	Unknown *m/z* 533 (peak 30)	

commercial betamethasone dipropionate standard compound was prepared and injected into mass spectrometer to obtain fragments (Fig. 2D). In Fig. 2D, two loss of *m/z* 74.0371 and 74.0373 indicated two side chains of propionate (theoretical value = 74.0326). The corticosteroid core contributed to the fragments of *m/z* 319, 337, and 355. The proposed molecular structures of fragments were generated from Mass Frontier software (Thermo-fisher Scientific Inc.) Fig. 2D bore some similarity to Fig. 2A, such as *m/z* 185, 263, 279, 301, 319, 337, and 355, revealed the unknown compound (*m/z* 533) and betamethasone dipropionate were structure similar compounds. In Fig. 2A, two identical side chains were proposed as butyrate because two loss of *m/z* 88.0528 were observed (theoretical value = 88.0519). Therefore, the unknown *m/z* 533 was suggested to be methasone dibutyrate. In Fig. 2B, very similar loss of side chains of *m/z* 88.0531, 88.0527, and 88.0493 were observed, therefore, the unknown *m/z* 603 was suggested to be methasone tributyrate. Methasone derivatives were glucocorticoids included epimers, dexamethasone (α form) and betamethasone (β form). The structural difference between them was only the orientation of methyl group on carbon at position 16. Therefore, betamethasone and dexamethasone had very similar mass spectra [1]. We tried to do a literature search including ChemSpider and ChemicalBook by using the key words of beta/dexa-methasone dibutyrate and beta/dexa-methasone tributyrate. Unfortunately, none of the information was found. The methasone dibutyrate and tributyrate is first time reported in this work to the best of my knowledge.

3.2. Synthesis of reference compounds, dexa/beta-methasone derivatives

The purification of unknown corticosteroids from sample and then run NMR might be needed in order to confirm their structures. However, considering the accessibility and convenient, it might be easier to synthesize the reference compounds, dexamethasone di- and tri-butyrate, betamethasone di- and tri-butyrate, due to these compounds bore the same side chains. The synthesis process was referenced to an esterification reaction of steroid ester [3]. Dexa- and beta-methasone was used separately as starting materials. The corticosteroid alcohols were first

Table 3
MS spectra of dexa/beta-methasone tributyrate and unknown *m/z* 603 in sample.

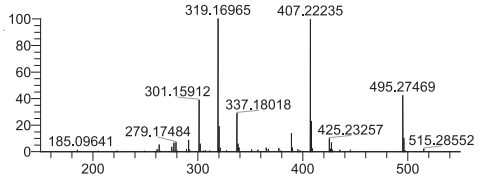
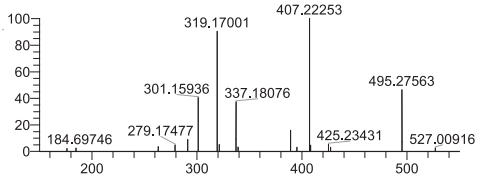
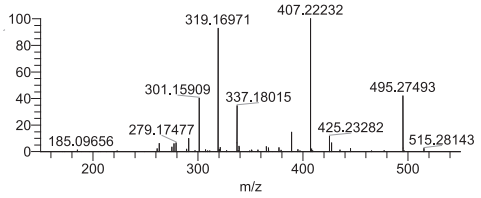
No	Name	Spectra
1	Dexamethasone tributyrate (peak 13)	
2		
	Betamethasone tributyrate (peak 27)	
3		
	Unknown *m/z* 603 (peak 31)	

Table 4
Average relative intensities and % standard deviations (n = 3) of the diagnostic ions of dexa/beta-methasone dibutyrate and unknown *m/z* 533 in sample.

Compound	533	425	355	337	319	407	(319 + 337)/355	319/355	319/407
Dexamethasone dibutyrate	26.8 ±3%	100.0	24.7 ±0%	37.7 ±3%	34.4 ±6%	11.3 ±11%	2.921	1.394	3.340
Betamethasone dibutyrate	28.1 ±2%	100.0	24.1 ±3%	36.4 ±2%	32.3 ±6%	14.5 ±0%	2.853	1.340	2.520
Unknown *m/z* 533	22.8 ±4%	100.0	27.0 ±1%	39.4 ±1%	36.7 ±2%	15.2 ±4%	2.813	1.357	2.594

Table 5
Average relative intensities and % standard deviations (n = 3) of the diagnostic ions of dexa/beta-methasone tributyrate and unknown *m/z* 603 in sample.

Compound	603	425	407	319	337	301
Dexamethasone tributyrate	100.0	43.7 ±1%	107.6 ±4%	97.8 ±0%	29.6 ±3%	40.1 ±7%
Betamethasone tributyrate	100.0	45.1 ±5%	100.7 ±5%	89.2 ±3%	29.1 ±3%	40.2 ±8%
Unknown *m/z* 603	100.0	43.0 ±5%	100.8 ±0%	90.1 ±2%	28.9 ±6%	39.9 ±1%

reacted with halogenomagnesium (Grignard reagent) to form steroid halogenomagnesium alkoxides, and then reacted with acid chlorides (butyryl chloride) to form the representative types of esters. This was a straightforward synthesis and the butyryl group was desired to be added at the 11, 17, and/or 21 positions. The reaction scheme was shown in Fig. 3. For methasone monobutyrate by using dexamethasone as an example, three products were expected as dexamethasone-11-butyrate, dexamethasone-17-butyrate, and dexamethasone-21-butyrate. For dexamethasone dibutyrate, three products were expected as dexamethasone-11, 17-dibutyrate, dexamethasone-11, 21-dibutyrate, and dexamethasone-17, 21-dibutyrate. For dexamethasone tributyrate, single product as dexamethasone-11, 17, 21-tributyrate was expected. Fig. 4A gave the chromatogram of synthesized dexamethasone derivatives. Apparently, the synthesis result was beyond our expectation. The peaks 1–4 were dexamethasone monobutyrate (SIM 463.2491, mass tolerance at 10 ppm). There were at least four relatively visible peaks instead of three for dexamethasone monobutyrate. The same phenomena were shown on the peak 5–10 which were dexamethasone dibutyrate (SIM 533.2909), and on the peak 11–15 which were dexamethasone tributyrate (SIM 603.3328). This might be due to the rotation of the 20-carbonyl derived side chain. Fig. 5 gave the possible rotational conformations. The results were similar to Rakhit's finding that the derived ketol side chain to the 20-carbonyl group of progesterone cannot rotate completely freely around the C_{17}-C_{20} single bond and that there existed four preferred conformations [10]. A similar phenomenon was also explained by cholestane 17-side chain that showed four principal conformations occurring in the ratio of 69:8:8:11 [13]. The products of synthesized betamethasone derivatives showed similar behavior, multi rotational isomers were observed in Fig. 4B. The esterification process through Grignard reaction applied in this study for synthesis of methasone derivatives was not a stereospecific reaction. This process led different stereoisomeric products, and the ratio of rational isomers was not controllable. The synthesis resulted similar product patterns but in different ratio in every batches. Besides, the isomers or called rotamers were subjected to small rotational transformations [10]. We observed that the predominant conformations transformed after storage at 4°C for 7 days in the same synthesis batch by comparing the stored compounds (Fig. 4C) with freshly synthesized compounds (Fig. 4A). Although the synthesis resulted multi-isomers which were not pure reference compounds, the individual compounds expressed different patterns of fragment spectra (see supplementary data for each spectrum of peak 1 to 31). Fig. 4D was sample chromatogram processed at SIM 533.2909 and 603.3328. The peak 30 at the retention time (RT) 10.74 min indicated unknown corticosteroids *m/z* 533. The LC condition in Fig. 4 was slightly different than Fig. 1 due to a slower gradient and higher initial

solvent content were applied in order to well separate the synthesized products, isomers and rotamers. In Fig. 4A, peak 10 matched the RT of unknown *m/z* 533 peak (compared to Fig. 4D) which were dexamethasone dibutyrate. In the other hand, in Fig. 4B, peak 25 also matched the RT of unknown peak (compared to Fig. 4D) which were betamethasone dibutyrate. The mass spectra of peak 30 in Fig. 4D, peak 10 in Fig. 4A, peak 25 in Fig. 4B were listed in Table 2. Apparently, the mass spectra in the sample matched our synthesized reference compounds, but we were not able to differentiate either dexa- or beta-methasone dibutyrate. For unknown *m/z* 603 in sample, we observed a single peak at RT 13.40 min in Fig. 4D. However, we also observed a match RT in Fig. 4A (peak 13, RT = 13.42) and Fig. 4B (peak 27, RT = 13.41) which indicated the tentative identification of dexamethasone tributyrate or betamethasone tributyrate. Their mass spectra of individual compounds were listed in Table 3. Apparently, the unknown *m/z* 603 matched both dexa- and beta-methasone tributyrate. Further investigation was needed to differentiate the real form, alpha- or beta-methasone in the sample.

3.3. Differentiation of dexamethasone and betamethasone derivatives

Betamethasone and dexamethasone were isomers differing only in the orientation of the methyl group on the C16 position. The discerning method for identifying these epimers was not found in the literature. However, some rough approaches had been successfully applied. Wasch [12] successfully differentiated dexamethasone and betamethasone by plotting the product ion 355 against the sum of 337 and 319 of a mixture of 0, 20, 40 to 100% of each combination of the α and β epimers. Detection was carried out by LC/MS positive ion mode with 25% collision energy. Arthur [1] studied epimers of betamethasone, dexamethasone, and various their esterification products by ESI-MS. The fragment ions (collision energy = 25 eV) at *m/z* 279 and 277 showed significantly different abundances in the two epimers as well as in their eaters. Other ions such as *m/z* 237, 161, and 121 were proposed to be the characteristic ions for betamethasone or the dexamethasone series. Deventer [2] identified the mixture of betamethasone and dexamethasone by the relative intensities of the diagnostic ions *m/z* 307, 325, and 345. Mass spectral data was detected in ESI negative mode with precursor ion 451 $(M + acetate)^-$ and collision energy 21.5% of maximum. Comparing the MS spectra of dexa- and betamethasone dibutyrate in our study, Table 2 showed almost identical spectra. Their diagnostic ions with relative intensities were summarized in Table 4. The discrimination between dexa and beta forms through the relative intensities of diagnostic ions was insufficient. However, sum of 319 and 337 then divided by 355 resulting in a difference in ratio [12]. In addition, the ratio of 319/407 showed significant difference between dexa and beta forms. Therefore, the unknown *m/z* 533 was more close to betamethasone dibutyrate. The relative intensities of diagnostic ions for synthesized dexa- and betamethasone tributyrate were summarized at Table 5. The fragments of *m/z* 319 and 407 showed different ion ratio between dexa and beta forms and could be used as a marker for discrimination. Therefore, the unknown *m/z* 603 was identified as betamethasone tributyrate.

4. Conclusion

Counterfeit drugs or drug analogues have been encountered in many countries in various products. Two new artificial synthesized corticosteroids called betamethasone dibutyrate and betamethasone tributyrate are identified in a food product. The safety and the drug effect of these two corticosteroid derivatives are unknown. Screening of new drug analogues or any new synthesized compound in a product is almost impossible by the contemporary triple quadruple mass spectrometry based methods which are popular for multiresidue analysis of pesticide and veterinary drug screening, because they are limited to preselected transitions. In this study the unknown compounds are initially doubted by conventional photodiode array spectra, and then high resolution mass spectrometry is applied for analysis. The MS fragments spectra of unknowns are compared to various reference compounds to elucidate the suspicious corticosteroids. The proposed candidates are also synthesized for confirmation. The alpha (dexa) and beta forms of corticosteroid could express different actions on its target [11]. The new found methasone derivatives are differentiated by discriminating their ion ratio of fragments. As the results, betamethasone dibutyrate and betamethasone tributyrate are confirmed and the mass spectra of fragments are reported for reference.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.steroids.2020.108739.

References

[1] K.E. Arthur, J. Wolff, D.J. Carrier, Analysis of betamethasone, dexamethasone and related compounds by liquid chromatography/electrospray mass spectrometry, Rapid Commun. Mass Spectrom. 18 (2004) 678–684.
[2] K. Deventer, F.T. Delbeke, Validation of a screening method for corticosteroids in doping analysis by liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Spectrom. 17 (2003) 2107–2114.
[3] D.D. Evans, D.E. Evans, G.S. Lewis, P.J. Palmer, D.J. Weyell, A novel method for the preparation of steroid esters, J. Chem. Soc. (1963) 3578–3582.
[4] M. Fang, C.F. Tsai, D.Y. Wang, H.F. Cheng, Structure elucidation of a novel analogue of pesticide from express post parcel by HRMS, SN Appl. Sci. 1 (2019) 1603.
[5] J. Fiori, V. Andrisano, LC-MS method for the simultaneous detection of six glucocorticoids in pharmaceutical formulations and counterfeit cosmetic products, J. Pharm. Biomedical Anal. 91 (2014) 185–192.
[6] M. Fiori, E. Piersominici, F. Longo, G. Brambilla, Identification of main corticosteroids as illegal feed additives in milk replacers by liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry, J. Chrom. A. 807 (1998) 219–227.
[7] V. Giaccone, G. Polizzotto, A. Macaluso, G. Cammilleri, V. Ferrantelli, Determination of ten corticosteroids in illegal cosmetics by a simple, rapid, and high-performance LC-MS/MS method, Int. J. Anal. Chem. 2017 (2017) 1–12.
[8] F. Guan, C. Uboh, L. Soma, A. Hess, Y. Luo, A.S. Tseng, Sensitive liquid chromatographic/tandem mass spectrometric method for the determination of beclomethasone dipropionate and its metabolites in equine plasma and urine, J. Mass Spectrom. 38 (2003) 823–838.
[9] P. Jin, X. Liang, X. Wu, X. Je, Y. Kuang, X. Hu, Screening and quantification of eighteen glucocorticoid adulterants from herbal pharmaceuticals and health foods by HPLC and confirmed by LC-Q-TOF-MS/MS, Food Addit. Contam. Part A. 34 (2017) 10–19.
[10] S. Rakhit, C.R. Engel, The conformations of the 20-keto side chain of C_{21}-steroids, Can. J. Chem. 40 (1962) 2163–2170.
[11] L. San Feliciano, A. Remesal, M. Isidoro-Garcia, D. Ludena. Dexamethasone and betamethasone for prenatal lung maturation: differences in vascular endothelial growth factor expression and alveolarization in rats. Neonatology, 100 (2011) 105–110.
[12] K.D. Wasch, H.F. De Brabander, M. Van de Wiele, J. Vercammen, D. Courtheyn, S. Impens, Differentiation between dexamethasone and betamethasone in a mixture using multiple mass spectrometry, J. Chrom. A 926 (2001) 79–86.
[13] L.D. William, F.G. Jane, C.R. Douglas, M.W. Charles, Conformational analysis of sterols: Comparison of X-ray crystallographic observations with data from other sources, Lipids 15 (9) (1980) 783–792.