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Rapid determination and quantification of hair-growth compounds in adulterated products by ultra HPLC coupled to quadrupole-orbitrap MS

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Abstract Recently, a number of adulterated products, which are advertised as hair-growth enhancer have been emerged among those who suffer hair loss disease. For continuous control of illegal products, in this study, a rapid and sensitive method for simultaneous screening of 12 compounds that enhance hair-growth was established to protect public health by ultrahigh-performance liquid chromatography coupled to quadrupole-orbitrap mass spectrometry (UHPLC-Q-Orbitrap-MS). Fragmentation pathways of them were proposed based on MS^2 spectral data obtained using the established method. In this analysis, the LODs and LOQs ranged from 0.05 to 50 ng/mL and from 0.17 to 167 ng/mL, respectively. The square of the linear correlation coefficient (R^2) was determined as more than 0.995. The intra- and inter-assay accuracies were respective 88-112 % and 88-115 %. Their precision values were measured within 5 % (intra-day) and 10 % (inter-day). Mean recoveries of target compounds in adulterated products ranged from 84 to 115%. The relative standard deviation of stability was less than 12 % at 4 °C for 48 h. The method was employed to screen 14 dietary supplements advertised to be effective for the treatment of hair loss. Some of the products (~21 %) were proven to contain synthetic drugs that promote hair growth such as triaminodil, minoxidil, and finasteride.

Key words: hair loss, adulterants, UHPLC-Q-Orbitrap-MS, screening, validation, fragmentation pathway

1. Introduction

Hair loss has been one of the serious problems in life quality. The market of the products for the treatments of hair loss thus grows rapidly. Several anti-alopecia drugs have been used orally or topically to alleviate hair loss and promote hair growth.[1-2] Some of the drugs such as finatsteride and minoxidil have been approved by the United States Food and Drug Administration (US FDA).[3-4] It has been believed that 5α-reductase, which converts testosterone into dihydrotestosterone (DHT), plays an important role

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in hair loss.[5] Finasteride, a 5α-reductase inhibitor, impedes scalp hair follicle miniaturization by lowering the blood level of DHT. Minoxidil, a vasodilator, enhances hair growth by providing hair follicles with more oxygen and nutrients.[6,7] Several other drugs have also been prescribed for the treatments of hair loss by approval of authorities in some countries and used off-label worldwide. Dutasteride reduces the formation of DHT by inhibiting 5α-reductase.[8] Spironolactone, cyproterone acetate and flutamide bind androgen receptors in competition with DHT.[9-10] Not only scalp hairs but facial hairs including beards, moustaches, sideburns, and eyebrows are also considered as important factors for good outward appearances. Contrary to scalp hair loss, whiskers, moustaches, and sideburns are stimulated to grow by testosterone, and androgenic-anabolic steroids (AAS) has thus been used for their growth.[11,12] In addition, the cosmetic formulation of bimatoprost, a synthetic prostaglandin analogue (PGA), to lengthen eyelashes was approved by US FDA and in high demand for women.[13-14]

A great number of products have been advertised and sold for the treatments of hair loss in dietary supplements. Consumers believe that these products are safe and do not contain any synthetic drugs or their analogues. However, several dietary supplement containing synthetic hair loss remedies have been manufactured and distributed illegally via the internet and black market without any toxicological declaration on their labels.[15-16] Abuse and misuse of the products could pose a significant risk to public health due to diverse side effects of their adulterants.[3,17] Therefore, routine screening of these products should be performed to protect consumers from the risk of illegal adulterants.

In recent years, analytical methods for detection of drugs that reverse hair loss have been developed by using capillary electrophoresis (CE), high performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS/MS).[13,18-23] In our laboratory, LC-MS method for determination of hair-growth compound were developed and applied to screen a total of 76 products advertised as hair growth enhancer.[24] However, a method for simultaneous identification of active substances that promote hair growth using high resolution mass spectrometry (HR-MS) has yet to be established with high specificity and accuracy.

The aim of present study was to develop a new UPLC-Q-Orbitrap-MS based method that can simultaneously screen 12 hair growths to identify their specific fragmentation pathway and screen illegally manufactured products for hair growth. The Q-Orbitrap-MS, as one of HR-MS group, is frequently applied for screening (non)-targeted compound in complex matrices using MS^2 spectra. Recently, new analogue of minoxidil were detected in adulterated dietary supplement by Q-Orbitrap-MS, which was identified as triaminodil.[25] Since new analogue is synthesised through minor chemical modification of the parent structures in an attempt to avoid their detection by authorities, we established fragmentation pathways for 12 hair growth compounds using MS^2 spectra of Q-Orbitrap-MS. This method was validated and applied to screen 14 dietary supplement advertised to be effective for the treatment of hair loss.

2. Experimental

2.1. Chemicals and reagents

Triaminodil was synthesized in our laboratories following a synthetic procedure for minoxidil in the literature.[26] In the synthesis of triaminodil, pyrrolidine was used instead of piperidine. Six standard compounds (alfatradiol, dutasteride, finasteride, flutamide, minoxidil, spironolactone and testosterone propionate) were purchased from USP (Rockville, MD, USA). Cyproterone acetate and diphenylcyclopropenone were supplied by Sigma-Aldrich (St, Louis, MO, USA). Bimatoprost and methyltestosterone were obtained from BOC Sciences (New York, USA) and TCI (Tokyo, Japan), respectively. Standard stock solutions (1000 μg/mL) were prepared in methanol and stored at 4 °C. Deionised water was prepared by using a Milli-Q-water purification system (Millipore, Billerica, MA, USA) at 18.2 MΩ cm^{-1}. Ammonium acetate was supplied by Biopure (Cornwall, UK). Methanol (MeOH) and acetonitrile (ACN) for the

HPLC grade were purchased from Merck KGaA (Darmstadt, Germany). All solvents in this study were HPLC grade and were filtered through a poly (vinylidenedifluoride) (PVDF) filter (0.2 μm).

2.2. Sample preparation

All 14 dietary supplements, advertised as hair growth enhancers, were obtained from online and offline markets in the last two years. They were in the form of capsules, tablets, powders, liquids. About 1 g of a homogenized sample was dissolved in 50 mL methanol and degassed in a sonication bath for 30 min. The extract was then centrifuged for 10 min at 3000 rpm to remove any extra matrix materials. An aliquot of the supernatant was filtered through a 0.2 mm polytetrafluoroethylene (PTFE) filter and diluted to appropriate concentration with MeOH for subsequent instrumental analyses.

2.3. UHPLC-Q-Orbitrap-MS analysis

The UHPLC-Q-Orbitrap-MS experiments were conducted by using a Q Exactive Orbitrap mass spectrometer equipped with a Thermo Dionex UltiMate 3000 LC (Thermo Scientific, San Jose, CA). The eluents were 1 mM ammonium acetate in distilled water (A) and acetonitrile (B), and the column was a Poroshell 120 EC-C_{18} (100 × 2.1 mm, i.d. 2.7 μm) maintained at 30 °C. The injected sample volume was 1 mL, and the flow rate was 0.4 mL·min^{-1}. After eluent B was initially maintained at 10 % for 2 min, the gradient profile was as follows: 2.0-6.0 min (A: 90-0 %, B: 10-100 %), 6.0-8.0 min (A: 0 %, B:100 %), 8.0-8.1 min (A: 0-90 %, B: 100-10 %), and 8.1-10.0 min (A: 90 %, B:10 %). Full MS/$ddMS^2$ (data-dependent MS^2) experiments were conducted. The mass spectrometer parameters were as follows: ion source, heated ESI (HESI); ion mode, positive[10] except for flutamide and alfatradiol; spray voltage, 3.5 kV(+) and 3.0 kV (–); capillary temperature, 320 °C; sheath gas, 42 arbitrary units; auxiliary gas, 10 arbitrary units; probe heater temperature, 350 °C (+) and 300 °C (–); S-lens RF level, 50; resolution, 70,000 (full scan), 17,500 (MS/MS); automatic gain control (AGC) target, $3e^6$ (full scan), $1e^5$ (MS/MS); scan range, 50 to 1000 *m/z*; maximum infusion time (IT), 100 ms (full scan) and 50 ms (MS/MS); microscans, 1; loop count, 5; MSX count, 1; Top N, 5; isolation window, 4 *m/z*; underfill ratio, 1.0 %; intensity threshold, $2e^4$; exclude isotopes, on; and dynamic exclusion, 10.0 s. The mass spectrometer was calibrated according to the manufacturer's instructions. Data were analysed by using Xcalibur 3.0 software (Thermo Scientific, San Jose, CA).

2.4. Method validation

Several analytical parameters of target compounds including specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, precision and accuracy, recovery, and stability were evaluated by running three replicates. The stock solution of each component was added to a 10 mL volumetric flask in an amount of 500 μL to prepare a 50 μg/mL working solution. The spiking process was performed by dissolving the three-type (solid, liquid, cream) matrix-blank sample in MeOH during the sample preparation and then adding the working solution appropriately to the concentration required for the analysis. The specificity was confirmed by comparing the blank with the spiked standards. LODs and LOQs of target compounds were determined using signal-to-noise (S/N) ratio of the lowest detectable concentration of standard compounds spiked in matrices, and defined as the analyte amount that yields respective *S/N* ratios 3 and 10. Linearity was evaluated using the square of the linear correlation coefficient (R^2) obtained by plotting the peak areas of six different concentrations. Serial dilutions for 10, 50, 100, 250, 500, and 1000 ng/mL were performed to obtain linearity except for alfatradiol. The linearity of alfatradiol was determined at 6 points of 200, 250, 500, 600, 750, and 1000 ng/mL. The inter- and intra-day parameters were also evaluated by performing three replicate experiments on three different days and in a day, respectively. Their accuracy was determined by comparing the average concentration calculated from the linear equation with the theoretical concentration. The precision was expressed by using the relative standard deviation (RSD). Percent recovery was calculated by

comparing the peak areas of an analyte and its spiked standard. Stability of the target compounds was evaluated for 24 h and 48 h at 4 °C.

3. Results and Discussion

3.1. Optimization of instrument conditions

To search the optimum compositions of mobile phase for chromatographic separation, several aqueous solutions were examined with ACN including 5 mM ammonium acetate, 1 mM ammonium acetate, 5 mM ammonium formate, and 0.1 % formic acid. We could not observe any significant differences in sensitivities of the target compounds in positive ion mode except for alfatradiol when 5 mM ammonium acetate, 5 mM ammonium formate, and 0.1 % formic acid solutions. Alfatradiol was resoluted better when 5 mM ammonium acetate solution was used compared to 5 mM ammonium formate and 0.1 % formic acid solutions. Peak tailings and sensitivities of all the compounds improved when 1 mM ammonium acetate solution was used. The results made us to choose 1 mM ammonium acetate in distilled water (A) and ACN (B) as the compositions of mobile phase for rapid and efficient chromatographic separation of the target compounds.

Several mass parameters were investigated to attain suitable selectivity and sensitivity for the target compounds. Considering that mass selectivity and sensitivity move in opposite directions according to full width half maximum (FWHM) values, the full MS resolution was optimized at 70,000 FWHM for the majority of the analytes to obtain suitable selectivity and sensitivity. Likewise, the dd-MS^2 resolution was set at 17,500 FWHM to get suitable selectivity and sensitivity. The mass tolerance window was within 5 ppm considering the detection capability, signal intensity, and matrix interferences. All mass errors for the protonated molecules ($[M + H]^+$) were ranged from -2.3 to 3.5 ppm, demonstrating that their mass accuracy was highly reliable in the UHPLC-Q-Orbitrap-MS (*Table* 1).

3.2. Fragmentation of hair growth

Fragmentations of 12 hair growth compounds were obtained from the full-MS/ddMS^2 of Orbitrap-MS which is composed of a full MS scan followed by 5 data-dependent scans with normalized collision energy

Table 1. Quasimolecular ions and MS^2 fragment ions of hair growth remedies

Compound	Quasimolecular ions formula	Retention time (min)	Quasimolecular ions (*m*/*z*)		Mass error (ppm)	Normalised collision energy (NCE)	MS^2 fragment ions (*m*/*z*)
			Calculated	Observed			
Triaminodil	$C_8H_{14}N_5O^+$	1.98	196.11929	196.11974	-2.3	37	179.10901, 151.08557, 137.08241, 110.05916
Diphenylcloprepenone	$C_{15}H_{11}F_6O^+$	5.40	207.08044	207.08089	-2.2	75	178.07793, 152.06221, 77.03945
Minoxidil	$C_9H_{16}N_5O^+$	4.02	210.13494	210.13519	-1.2	33	193.12468, 164.09337, 151.07764, 137.08244, 110.05927
Methyltestosterone	$C_{19}H_{29}O_2^+$	5.72	303.23186	303.23218	-1.1	38	109.06535, 97.06545
Spironolactone	$C_{24}H_{33}O_4S^+$	5.78	341.21112	341.21149	-1.1	34	187.11203, 107.08608
Testosterone propionate	$C_{22}H_{33}O_3^+$	6.88	345.24242	345.24121	3.5	35	253.19524, 109.06533, 97.06544
Finasteride	$C_{23}H_{37}N_2O_2^+$	5.61	373.28495	373.28506	-0.3	45	317.22238, 305.25885
Bimatoprost	$C_{22}H_{40}NO_5^+$	5.10	398.29010	398.29019	-0.2	45	362.24811, 317.19037, 275.17850, 131.08534, 91.05457, 117.06989
Cyproterone acetate	$C_{22}H_{28}ClO_3^+$	6.18	417.18271	417.18307	-0.9	23	357.16171, 313.13553, 279.17435, 147.11700, 133.10143
Dutasteride	$C_{27}H_{31}F_6N_2O_2^+$	6.42	529.22842	529.22900	-1.1	46	461.20267, 133.10143,69.03410, 95.08580
Flutamide	$C_{11}H_{10}F_3N_2O_3^-$	5.91	275.06490	275.06500	-0.4	41	202.01154, 182.00508, 175.02412
Alfatradiol	$C_{18}H_{23}F_3O_2^-$	5.46	271.17035	271.17045	-0.4	67	183.08083, 145.06482

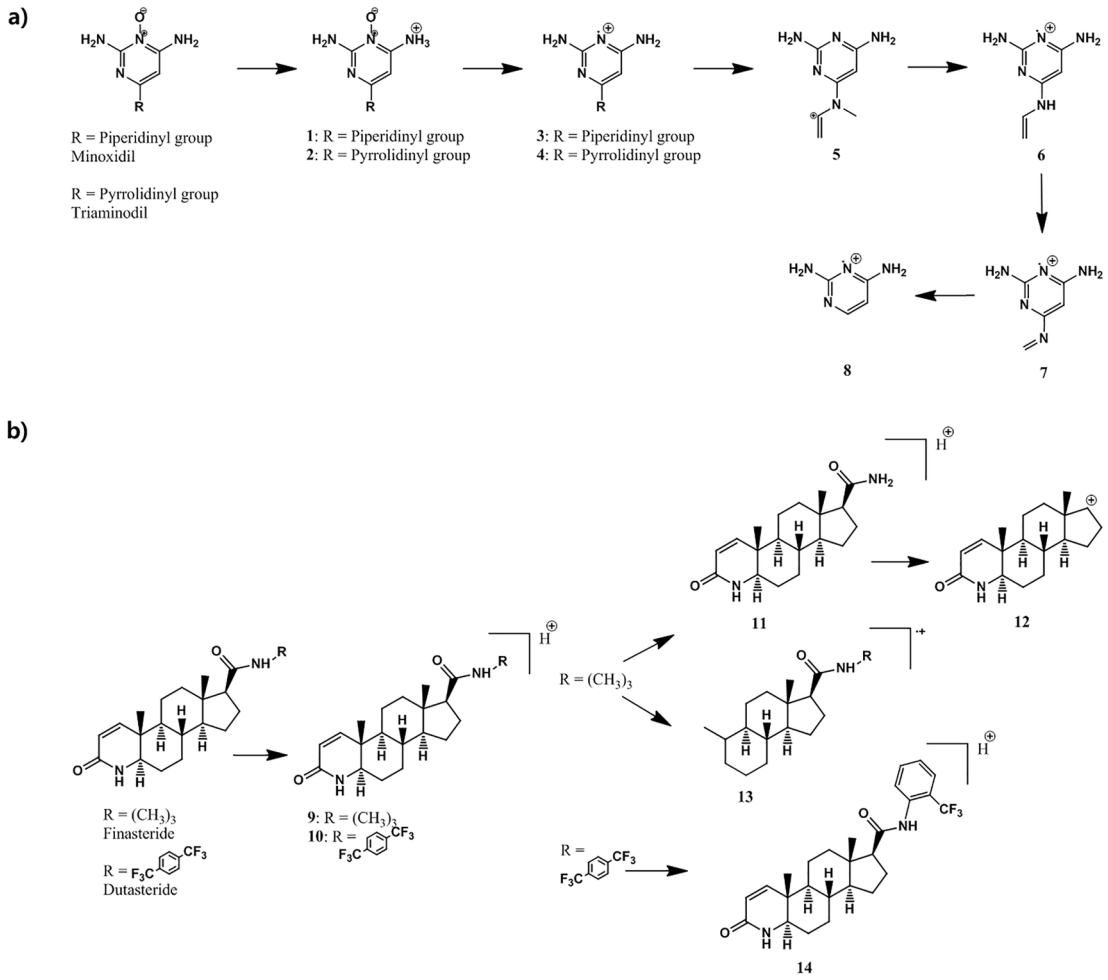

Fig. 1. Proposed fragmentation pathways for (a) minoxidil and triaminodil, (b) dutasteride and finasteride.

of 23-75 NCE. In *Table* 1, the characteristic fragment ions of significant abundance are donated for hair growth compounds. In order to better understand the observed fragmentation in the MS^2 spectrum, a fragmentation pathway is proposed for each compound as shown in *Figs.* 1 and 2. The fragmentation pathways were carefully envisaged using the following several factors in previous literature.[27] First, the carbon atom(s) with branches or strain conducted to inductive cleavage. Second, stability depends on carbocation order (tertiary > secondary > primary) and positive charge stabilized by resonance or inductive effects. Eight of 12 compounds were classified as chemical structure. Minoxidil and triaminodil had a similar fragment ion pattern (**1-8**) with a difference of 14 Da depending on the chemical of R, which is described by the sequential cleavage in the R structure. Fragmentation pathways (**9-14**) of finasteride and dutasteride were distinguished by their amide group. Methyltestosterone and testosterone propionate were found to produce common fragment patterns (**15-22**), eliminating R_1, R_2, ketone, and methyl group. Cyproterone acetate and spironolactone had fragment patterns depend on R_1 and R_2. Cyproterone acetate generated specific ions **25** to **27** because of sequential breaks of acetyl, methyl and ketone, and chloride, whereas spironolactone showed **28** to **30** from ions m/z 341 $[M\text{-}SCOCH_3]^+$ which is dominated with higher

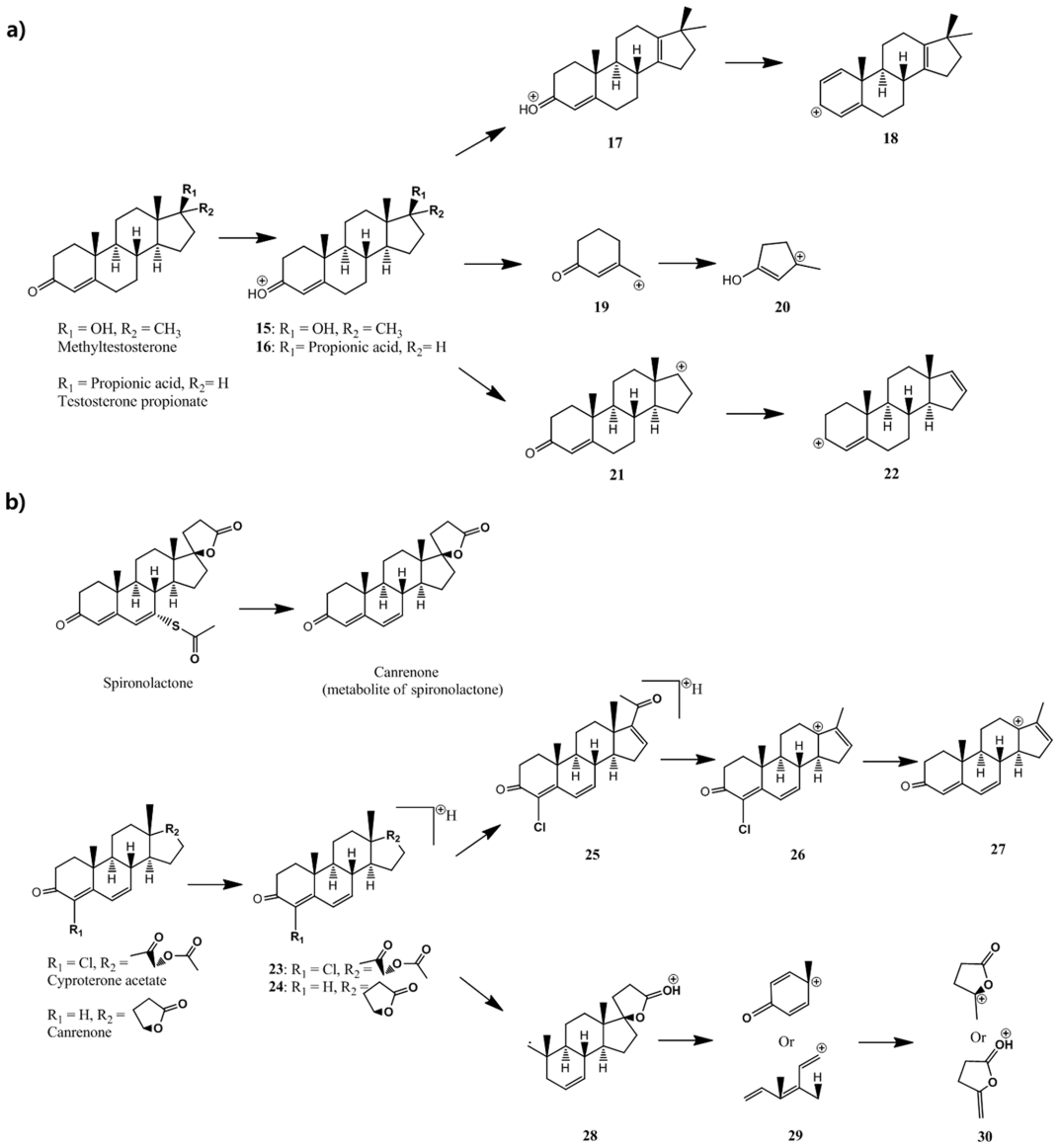

Fig. 2. Proposed fragmentation pathways for (a) methyltesto-sterone and testosterone propionate, (b) cyproterone acetate and spironolactone.

intensity and stability. Fragmentation process of alfa-tradiol, bimatoprost, diphencyclopropenone, flutamide were proposed in *Fig.* S1, respectively. These fragment peaks can be possibly used as a transition in the MRM analysis.

3.3. Method validation

Specificity was investigated by comparing several types of matrix blanks and matrix spikes for the 12 target compounds. As shown in *Fig.* 3, no interference peaks were observed in the chromatograms. We thus concluded that our UHPLC-Q-Orbitrap-MS experiments could provide a resolution high enough to distinguish the analytes from their isobaric ions. Several analytical parameters for the target compounds are indicated in *Table* S1 including LODs, LOQs, and linearity. LODs and LOQs for the target compounds in three types of matrices were determined as the lowest

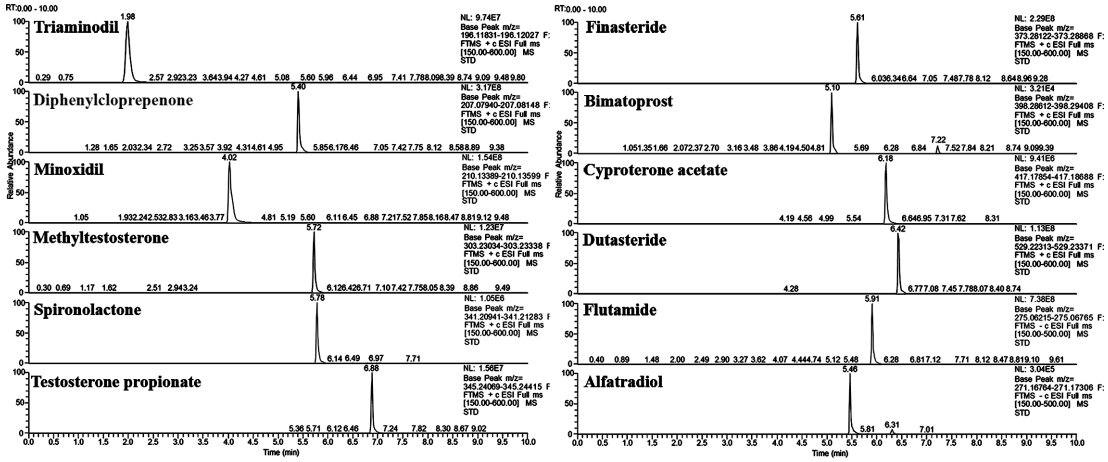

Fig. 3. The extracted parent ion chromatograms of target compounds spiked in solid type matrix-blank sample by using UHPLC-Q-Orbirap-MS.

concentrations yielding S/N ratios of 3 and 10, respectively. LODs were in range of 0.05-50 ng/mL for three types of matrices, and the range of LOQs was from 0.17 to 167 ng/mL. The linearity was evaluated by plotting the peak area corresponding to six serial concentrations ranging from 10 to 1000 ng/mL. All the R^2 values for the target compounds were higher than 0.995. The value indicates highly good linearity of the method. Accuracy and precision were assessed at three concentrations (low, medium, and high) for intra- and inter-day comparisons. Accuracy was determined by the percent recovery while precision was evaluated with the inter-day repeatability and reproducibility using the relative standard deviation (RSD). The intra- and inter- day accuracy ranged from 88 to 112 % and 88 to 115 %, respectively (*Table* S2). The precision was within 5 % (intra-day) and 12 % (inter-day).

As shown in *Table* S3, mean recoveries of the target compounds from the three types of matrices were in ranges of 85-104 % (solid), 86-112 % (liquid), and 84-115 % (cream). In addition, their precisions were less than 6 %. These results demonstrate that this newly developed method is highly efficient to analyze the target compounds with relatively low matrix effects. Stability was examined at different

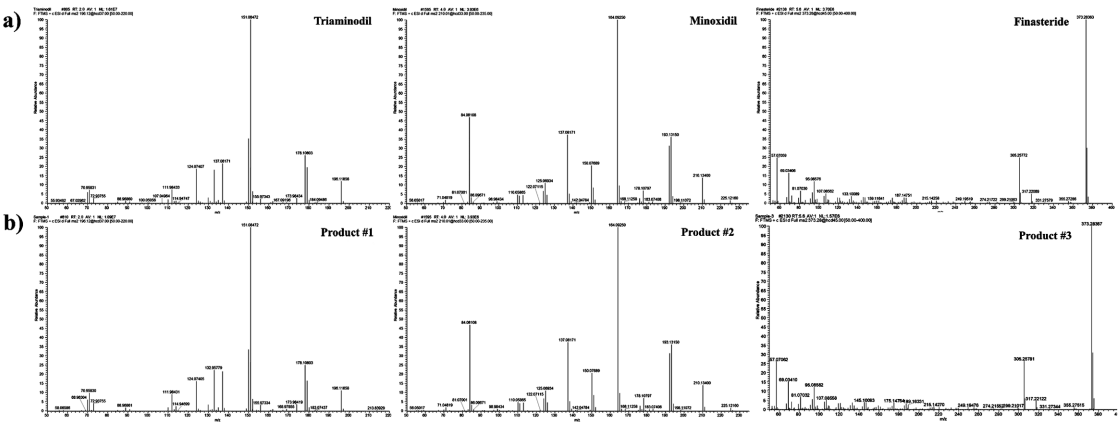

Fig. 4. UHPLC-Q-Orbirap-MS/MS product ion spectra (high resolution) of triaminodil, monoxidil, and finasteride in (a) the standard solutions and (b) the adulterated products.

Table 2. Detected numbers of dietary supplements (n=14)

Sample	Detected compound	Amount (mg/g)
Solid-1	*n.d.	-
Solid-2	n.d.	-
Solid-3	Minoxidil	5.9
Solid-4	n.d.	-
Solid-5	n.d.	-
Solid-6	n.d.	-
Solid-7	Finasteride	13.4
Solid-8	n.d.	-
Solid-9	n.d.	-
Solid-10	n.d.	-
Solid-11	Triaminodil	16.4
Liquid-1	n.d.	-
Liquid-2	n.d.	-
Liquid-3	n.d.	-
Total	5	5.9-16.4

*n.d. not detected

storage times at 4 °C. Each RSD was within 8 % (24 h) and 12 % (48 h), which indicates that all target compounds were stable during UHPLC-Q-Orbitrap-MS experiments (*Table* S4).

3.4. Sample application

Several types of 14 dietary supplements, advertised as hair loss remedies, were rapidly screened by UHPLC-Q-Orbitrap-MS. The target compounds were detected within 10 ppm mass tolerance and further confirmed by performing MS/MS experiments (*Fig.* 4). As summarized in *Table* 2, approximately 21 % of samples (3/14) were adulterated with triaminodil, finasteride, and minoxidil in solid-type. The retention time of detected compounds were ranged from 1.9 to 5.6 min and mass accuracies were < 4.5 ppm, which indicated positive results. Diagnostic fragment ions of samples corresponded to those of standard solutions. The amounts of the drugs or a drug analogue in the adulterated products ranged from 5.9 to 16.4 mg/g, which indicated the possibility of considerable risk to the health of public.

4. Conclusions

A rapid and sensitive method for simultaneous identification of 12 drugs or their analogues for the treatments of hair growth in dietary supplement was established by UHPLC-Q-Orbitrap-MS/MS. Specific fragmentation pathways were proposed by interpreting the MS^2 spectra of protonated ions. We have demonstrated the accuracy and practicality of the method by identifying 3 adulterated products from 14 products. Several drugs and a drug analogue were detected in adulterated products including minoxidil, triaminodil, and finasteride in amounts that ranged from 5.9 to 16.4 mg/g. These adulterated products contained large amounts of drugs and a drug analogue enough to cause serious side effects. This newly developed method will thus be highly useful for authorities to screen dietary supplements advertised as hair loss remedies. Also, the results of this fragmentation study may be useful monitoring for rapid identification of new substances, which should contribute to efforts to safe guard food safety and public health.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Supplementary data

Rapid determination of hair-growth compounds in adulterated products by quadrupole-orbitrap mass spectrometry.

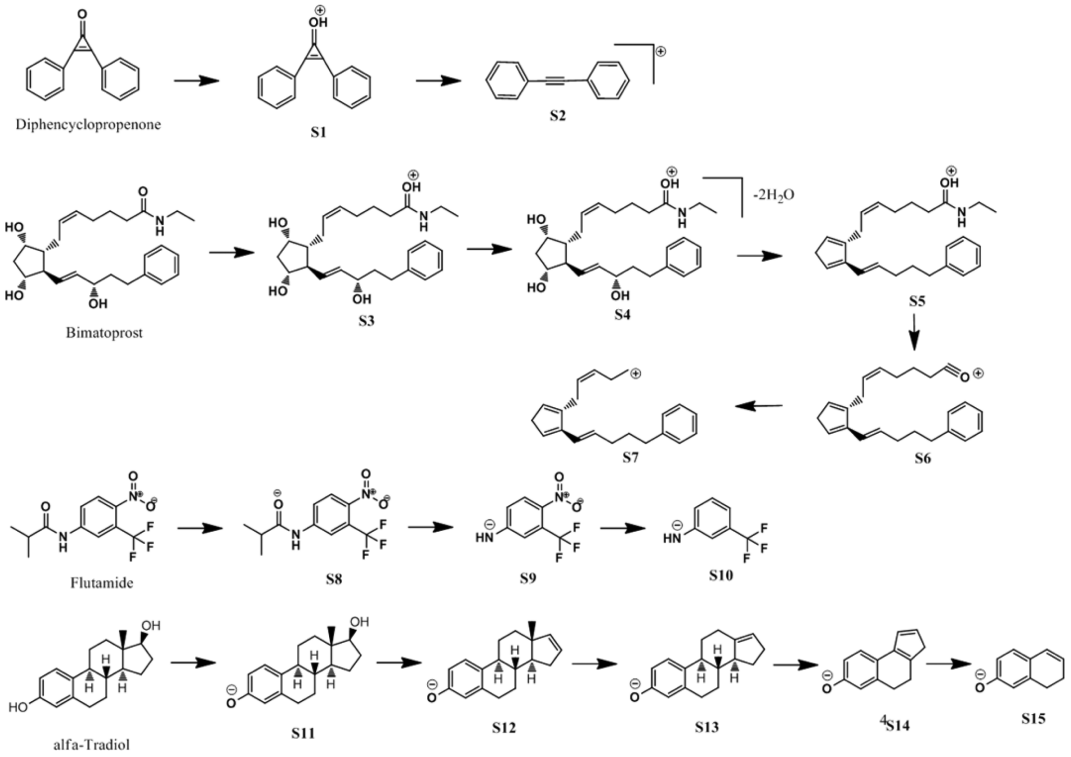

Fig. S1. Proposed fragmentation pathways for hair growth remedies.

Table S1. LODs, LOQs and linearity of hair growth remedies (n = 3)

Compounds	Range (ng/mL)	r^2	Solid		Liquid		Cream	
			LOD (ng/mL)	LOQ (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
Triaminodil	10-1000	0.995	0.42	1.40	0.21	0.70	0.21	0.70
Diphenylcycloprepenone	10-1000	0.997	0.08	0.27	0.54	1.80	0.08	0.27
Minoxidil	10-1000	0.998	0.52	1.73	0.26	0.87	0.52	1.73
Methyltestosterone	10-1000	0.999	2.49	8.30	1.00	3.33	1.00	8.33
Spironolactone	10-1000	0.997	2.45	8.17	2.45	8.17	2.45	8.17
Testosterone propionate	10-1000	0.999	0.99	3.30	2.48	8.27	2.48	8.27
Finasteride	10-1000	0.998	0.08	0.25	0.08	0.25	0.08	0.25
Bimatoprost	10-1000	0.999	0.50	1.67	0.25	0.83	0.25	0.83
Cyproterone acetate	10-1000	0.997	0.53	1.77	1.06	3.53	0.27	0.90
Dutasteride	10-1000	0.998	0.11	0.37	0.27	0.90	0.11	0.37
Flutamide	10-1000	0.999	0.05	0.17	0.05	0.17	0.05	0.17
Alfatradiol	200-1000	0.998	50.00	166.67	50.00	166.67	50.00	166.67

Table S2. Intra- and inter-day assay precision and accuracy for hair growth remedies (n=3)

Compounds	Conc. (ng/mL)	Intra-day		Inter-day	
		Accuracy (%)	Precision (RSD%)	Accuracy (%)	Precision (RSD%)
Triaminodil	10	96.33	1.52	99.65	0.95
	100	110.43	1.93	114.80	4.70
	1000	95.94	4.04	94.39	5.20
Diphenylcloprepenone	10	94.67	3.29	97.88	3.89
	100	96.46	5.00	109.72	5.55
	1000	89.26	1.92	93.57	4.80
Minoxidil	10	108.12	3.81	105.46	2.65
	100	96.32	3.79	97.26	3.06
	1000	97.14	3.49	97.14	5.53
Methyltestosterone	10	95.15	3.53	98.06	6.18
	100	88.45	0.76	102.33	6.39
	1000	89.12	3.48	95.16	9.89
Spironlactone	10	102.47	2.79	111.87	2.25
	100	90.87	2.72	95.17	6.50
	1000	91.18	3.91	90.50	6.86
Testosterone propionate	10	111.59	1.91	110.36	2.90
	100	92.95	3.19	98.24	8.36
	1000	91.80	2.41	97.23	2.73
Finasteride	10	101.07	1.01	100.41	5.10
	100	99.91	2.75	102.83	5.68
	1000	97.02	4.20	98.96	8.61
Bimatoprost	10	101.34	4.14	97.19	4.76
	100	101.67	1.46	95.68	5.56
	1000	102.51	1.47	94.44	8.25
Cyproterone	10	101.12	3.83	101.12	4.83
	100	105.48	4.58	104.66	1.68
	1000	103.00	4.22	91.50	11.61
Dutasteride	10	99.89	4.91	104.63	5.18
	100	101.08	2.28	105.36	7.56
	1000	108.41	3.68	96.14	10.10
Flutamide	10	102.72	1.93	108.25	4.74
	100	109.23	4.33	105.22	3.30
	1000	105.77	3.83	105.47	4.53
Alfatradiol	200	98.82	1.65	93.62	5.11
	500	95.70	1.66	87.93	8.14
	1000	103.74	1.81	95.01	8.25

T*able* S3. Recoveries of hair growth remedies spiked in three types of matrices (n=3)

Compounds	Conc. (ng/mL)	Solid		Liquid		Cream	
		Recovery (%)	Precision (RSD%)	Recovery (%)	Precision (RSD%)	Recovery (%)	Precision (RSD%)
Triaminodil	10	91.31	2.31	102.66	2.80	100.94	1.23
	100	85.54	0.94	98.33	2.94	98.37	0.20
	1000	100.13	0.91	96.82	0.47	98.87	0.45
Diphenylcloprepenone	10	95.03	1.54	107.88	4.61	113.13	3.12
	100	93.12	2.01	109.36	3.32	99.62	1.19
	1000	96.34	0.34	97.33	1.95	93.22	0.58
Minoxidil	10	102.52	4.20	108.06	0.81	99.48	2.01
	100	99.20	1.25	109.94	2.61	94.94	0.54
	1000	104.12	0.97	99.77	1.12	90.26	3.31
Methyltestosterone	10	95.74	3.55	86.29	2.05	87.47	5.42
	100	92.57	3.29	104.95	2.68	100.23	0.66
	1000	98.07	0.32	99.45	2.62	100.18	3.82
Spironlactone	10	85.71	0.00	85.71	0.00	85.71	0.00
	100	95.61	2.24	98.19	5.92	85.27	0.00
	1000	100.13	0.25	97.75	3.16	98.31	2.14
Testosterone propionate	10	91.67	2.89	105.00	5.00	88.33	2.89
	100	91.48	1.82	112.37	2.52	101.86	1.46
	1000	91.51	0.39	90.16	3.09	100.19	1.94
Finasteride	10	97.96	3.29	110.23	3.92	112.13	2.59
	100	96.75	1.20	108.50	5.18	97.28	1.81
	1000	99.59	0.39	99.99	2.38	98.40	0.85
Bimatoprost	10	89.45	0.57	100.53	0.29	101.53	0.76
	100	87.02	5.09	100.20	3.78	83.92	0.24
	1000	104.12	0.86	100.40	2.06	94.65	1.48
Cyproterone	10	91.60	0.00	94.15	5.83	104.33	5.83
	100	90.02	1.68	88.04	2.26	88.04	4.28
	1000	100.35	1.73	94.52	4.41	94.51	1.16
Dutasteride	10	92.35	3.40	102.60	5.80	112.24	0.36
	100	91.58	2.97	103.56	2.98	100.01	0.61
	1000	101.78	3.15	100.09	3.03	102.17	1.61
Flutamide	10	99.71	3.26	93.48	1.86	101.17	1.08
	100	99.63	0.29	100.66	2.23	102.20	4.24
	1000	87.68	1.11	100.05	2.64	108.92	0.87
Alfatradiol	200	101.85	0.73	97.67	4.88	114.80	0.95
	500	99.54	5.69	105.64	1.99	115.25	0.52
	1000	84.76	2.39	103.36	1.10	107.13	0.76

Table S4. Stability of hair growth remedies over 48h (n=3)

Compounds	Conc. (ng/mL)	RSD (%)	
		24 h	48 h
Triaminodil	10	7.10	0.03
	100	3.94	7.55
	1000	0.45	0.98
Diphenylcloprepenone	10	1.56	2.34
	100	5.40	7.94
	1000	2.40	1.98
Minoxidil	10	7.78	1.20
	100	2.76	3.67
	1000	1.92	1.70
Methyltestosterone	10	4.71	0.00
	100	3.53	7.84
	1000	0.05	2.34
Spironlactone	10	5.36	5.19
	100	7.65	3.56
	1000	7.40	2.39
Testosterone propionate	10	0.66	1.97
	100	5.53	2.09
	1000	2.91	0.79
Finasteride	10	8.38	11.61
	100	4.07	5.44
	1000	2.83	5.74
Bimatoprost	10	4.60	4.49
	100	4.19	1.88
	1000	2.14	6.25
Cyproterone	10	1.72	9.53
	100	5.64	0.27
	1000	4.07	3.28
Dutasteride	10	3.93	8.98
	100	0.77	1.70
	1000	1.91	0.11
Flutamide	10	2.28	6.51
	100	4.13	3.86
	1000	2.80	3.54
Alfatradiol	200	2.80	7.78
	500	4.25	11.68
	1000	3.27	8.09