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Application of an ultrahigh-performance liquid chromatography coupled to quadrupole-orbitrap high-resolution mass spectrometry for the rapid screening, identification and quantification of illegal adulterated glucocorticoids in herbal medicines

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ABSTRACT

In this work, the ultrahigh-performance liquid chromatography quadrupole orbitrap high resolution mass spectrometry (UHPLC-Q-Orbitrap HRMS) was applied to the rapid screening, identification and quantification of the illegal adulterated glucocorticoids in herbal medicines. The mass spectrometer was operated in positive ion mode and Full MS/dd-MS^2 (data-dependent MS^2) mode, where selected ions were subjected to a dd-MS^2 scan with given fragmentation energy following a Full MS scan. The application of 70 000 FWHM mass resolution and narrow mass windows (5 ppm) effectively improve the selectivity of the method, and a single injection was sufficient to perform the simultaneous screening and identification/quantification of 14 glucocorticoids in 15 min. The method validation including selectivity, sensitivity, calibration curve, accuracy, precision, recovery, matrix effect and stability were evaluated. The results of all analytes showed excellent linear relationship while all coefficient of determination (r^2) were > 0.9990 over wide concentration ranges (e.g., 5–1000 ng/mL for hydrocortisone butyrate, r^2 = 1.0000). The recoveries were in the range of 86.1–102.7%, while the matrix effects ranged from 95.8%–105.8%. Accuracies and precisions were performed. The intra- and inter-day accuracies ranged from 90.6% to 108.9%, while the intra- and inter-day precisions were in the range of 0.5% to 8.5%. Finally, the established method was employed to detect illegal adulterated glucocorticoids in herbal medicines. It will provide more reliable technical basis for the drug quality supervision department and ensure public health.

1. Introduction

Herbal medicines have been attracting high attention as they are believed to be safer and healthier than synthetic drugs [1]. In China, Traditional Chinese Medicines (TCMs) industry has developed rapidly in recent decades. Meanwhile, it has been confronted with new challenges. In pursuit of high business profits, some companies adulterated illegal chemical in TCMs, leading to series of seriously healthy and social problem.

Glucocorticoids are a class of corticosteroids which are used to treat diseases caused by an overactive immune system, such as allergies, asthma, autoimmune diseases, and sepsis, with their effects in the treatment of pain, inflammation, fever and rheumatism [2]. However, they have many pleiotropic effects including potentially harmful side effects such as osteoporosis, immunosuppression, increased skin fragility, etc. [3,4]. The risk of these side effects increases with long-term use and high doses, especially taken without medical supervision. Therefore, they are rarely sold over the counter and have been prohibited for adulteration into Chinese traditional medicines. Unfortunately, in the past several years, synthetic glucocorticoids have been routinely identified in so-called "all-natural" herbal medicines declared effects of antirheumatic and antitussive. Consequently, efficient and reli-

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Table 1
Molecular formula, retention time, accurate mass, mass error, NCEs and MS^2 fragment ions of fourteen glucocorticoids.

Analyte	Molecular formula	Retention time (min)	Protonated ion mass $[M+H]^+$		Mass error (ppm)	Normalised collision energy(NCE)	MS^2 fragment ions $[M+H]^+$
			Theoretical	Experimental			
Prednison	$C_{21}H_{26}O_5$	6.04	359.1853	359.1855	0.6	22	341.1751, 323.1649, 313.1795, 295.1693, 277.1592
Clobetasol Propionate	$C_{25}H_{32}ClFO_5$	11.97	467.1995	467.1995	0.0	12	373.1562, 355.1461, 156.3091
Hydrocortisone Acetate	$C_{23}H_{32}O_6$	11.19	405.2272	405.2259	−3.2	28	327.1962, 309.1859, 241.1583, 121.0652
Cortisone Acetate	$C_{23}H_{30}O_6$	11.35	403.2115	403.2107	−2.0	32	343.1913, 325.1794, 163.1120
TriamcinoloneAcetonide	$C_{24}H_{31}FO_6$	10.97	435.2177	435.2172	−1.1	14	415.2123, 397.2018, 357.1703, 339.1597, 321.1493
Hydrocortisone Butyrate	$C_{25}H_{36}O_6$	11.59	433.2585	433.2585	0.0	24	214.0901, 198.1861, 158.0282
Beclomethasone Dipropionate	$C_{28}H_{37}ClO_7$	12.16	521.2301	521.2300	−0.2	11	517.3215, 503.2208, 235.6592
Fluocinonide	$C_{26}H_{32}F_2O_7$	11.73	495.2189	495.2193	0.8	15	373.2772, 356.3887, 319.1317, 106.5421, 83.2636
Prednisolone	$C_{21}H_{28}O_5$	6.13	361.2010	361.2003	−1.9	10	340.6055, 233.0345, 120.3599
Betamethasone	$C_{22}H_{29}FO_5$	9.24	393.2072	393.2067	−1.3	10	373.2006, 355.1899, 376.2590, 337.1794
Triamcinolone	$C_{21}H_{27}FO_6$	3.44	395.1864	395.1861	−0.8	13	375.1807, 357.1702, 339.1596
Hydrocortisone	$C_{21}H_{30}O_5$	6.29	363.2166	363.2154	−3.3	31	327.1958, 309.1851, 267.1746, 121.0651
Prednisone Acetate	$C_{23}H_{28}O_6$	11.31	401.1959	401.1962	0.7	18	383.1866, 341.1764, 313.1817, 295.1693
Dexamethasone	$C_{22}H_{29}FO_5$	9.58	393.2072	393.2064	−2.0	13	373.2006, 355.1901, 337.1794, 376.2592

able methods should be developed urgently for the screening and identification of glucocorticoids.

The analytical methods hitherto developed for the detection of glucocorticoids include HPLC [5–7] and liquid chromatography–mass spectrometry (LC–MS) [8–14]. However, HPLC methods can hardly meet the requirement of simultaneous assay of various glucocorticoids, and its sensitivity was low. In most of the current LC–MS methods, the liquid chromatography is coupled with low resolution mass spectrometry (LRMS) analyzer such as ion trap (IT) [12], meanwhile, high resolution mass spectrometry (HRMS), such as quadrupole time-of-flight (Q-TOF) is rarely applied and mainly used in the analysis of biological matrices [8]. When mixture or unknown ingredients have to be identified in herbal medicines, LRMS is insufficient for unambiguous identification. Different from LRMS, the high resolution mass spectrometry (HRMS) could provide much higher mass accuracy, which benefits the high-resolution identification.

The Orbitrap is a new member of the HRMS analysers. It combines high speed with excellent quantification properties, ranking favourably in many analytical applications [15].The resolving power of Orbitrap is capable of in excess of 1 000 000 FWHM [16]. So far the Orbitrap technique has been applied for qualitative or quantitative analysis in various fields such as doping control, drugs of abuse, pesticide residues etc. [17–19]. This study aimed at developing a rapid screening method for the simultaneous identification, confirmation and quantification of 14 glucocorticoids in herbal medicines employing UHPLC coupled with Q-Orbitrap HRMS. To the best of our knowledge, this is the first time for the application of Orbitrap HRMS for the screening of multiple glucocorticoids.

2. Materials and methods

2.1. Chemicals and reagents

Prednison (98.50%), clobetasol propionate (99.8%), hydrocortisone acetate (100%), cortisone acetate (99.2%), triamcinolone acetonide (100%), hydrocortisone butyrate (100%), beclomethasone dipropionate (100%), fluocinonide (100%), prednisolone (99.4%), betamethasone (99.3%), triamcinolone (99.64%), hydrocortisone (100%), prednisone acetate (99.7%) and dexamethasone (99.7%) were purchased from National Institutes for Food and Drug Control (Beijing, China). Herbal medicines claimed that possessed anti-inflammatory, analgesic, anti-rheumatism or immunosuppression effects were collected by Shandong Food and Drug Administration. Herbal medicines without the detected fourteen glucocorticoids were used as blank matrices. HPLC grade methanol, acetonitrile and formic acid (FA, ≥99.0%) were purchased from TEDIA Inc. (USA). Ultrapure water (18.2 MΩ) was obtained from a Milli-Q Advantage A10 ultrapure water purification system.

2.2. Instrumentation

The UHPLC–HESI–Q-Orbitrap HRMS system is composed of an Accela 1250 LC pump and an Accela open autosampler coupled with a Q Exactive™ high resolution mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). XCalibur 2.2 software from Thermo Fisher Scientific (MA, USA) was used for controlling the instrument and processing data, while Q Exactive 2.1 (tune application) software (Thermo Fisher Scientific) for mass spectrometer control. Chromatographic separation was achieved on a Hypersil GOLD C_{18} chromatographic column (100 × 2.1 mm, 1.9 μm) (Thermo Fisher Scientific). All centrifugation was performed on a Sigma 3–30 K refrigerated centrifuge (Sigma, Steinheim, Germany). Ultrasonic