cological effects of semen pharbitidis is still insufficient, and further research is needed. It provides a favorable theoretical basis for the clinical application of Traditional Chinese medicine, and makes it serve the human health better.

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DEVELOPMENT OF A UPLC-MS/MS COMPOSITIONAL SUGAR ANALYTICAL METHOD TO DISCRIMINATE POLYSACCHARIDES FROM GENUS EPHEDRA

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ABSTRACT Mahuang is a famous traditional Chinese medicine, has been used for thousands of years for the treatment of allergies, asthma, pneumonia, bronchitis, hay fever and colds。 Ephedra sinica polysaccharides have been reported to possess important immunosuppressive activities, so quality evaluation of polysaccharides from genus Ephedrais extremely urgent. In this study, methods involving enzymatic digestions have been developed to establish multiple saccharide fingerprints through ultra-performance liquid chromatography with electrospray ionization triple quadrupole linear ion trap mass spectrometry (UPLC-ESI-TQ-LIT-MS/MS) based on a multiple-reaction monitoring in negative mode. Under optimum UPLC-MS/MS conditions, excellent separation and quantification of 22 constituents were achieved within 20 min on a solid core column with a 1.6 μm particle using pre-column derivatization with a PMP reagent. This method coupled with principal component analysis has been successfully applied to characterize and discriminate Ephedra polysaccharides attributed to different species and medical parts.

Key words: UPLC-MS/MS; polysaccharides; genus Ephedra; mild enzymatic digestion

Result In this work, a reliable, simple and sensitive PMP pre-column derivatization method was developed for the simultaneous analysis of 21 PMP derivatives characterized by the presence of 7 neutral sugars, 2 uronic acids, 3 amino sugars, 2 acetyl amino sugars, 6 oligosaccharides and 1 degradation product employing UPLC-ESI--TQ-MS/MS technique based on a solid core cortecs C18 column within 20 min. The proposed method was featured by minimizing sample handling and permitting high throughput analysis, and has been successfully applied to analyze 20 Ephedra polysaccharide samples from different species and medical parts. Multivariate statistical analysis results indicated that specific enzymatic digestions (α-amylase, β-(1→3)-D-glucanase and cellulose) could be further used for distinguishing these polysaccharides from genus Ephedra. The enzymatic digestions followed by UPLC-ESI--TQ-LIT-MS/MS coupled with multivariate statistical analysis may be a powerful and practical approach for comprehensive quality evaluation of plant polysaccharides from traditional Chinese medicines.

FRAGMENTATION PATTERN OF SPIROSTANOL STEROIDAL SAPONINS FROM ANEMARRHENA ASPHODELOIDES

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Abstract: Anemarrhena asphodeloides (A. asphodeloides) rhizome roots of liliaceous is widely used to clearing heat-fire, nourishing Yin and moistening dryness, and recorded in "Chinese pharmacopoeia" 2015 edition. So far, more than 50 steroid saponins have been isolated from A. asphodeloides, which is widely used to decrease blood glucose levels, inhibit platelet activity and carcinoma activity. This study applying UPLC-MS/MS methods to analyze Anemarrhena spirostanol reference standards. Finally, fragmentation regularities of four spirostanol steroid saponins are summarized. The four reference standards (RSs) are timosaponin AⅡ (1), timosaponin AⅢ (2), dioscin (3), ophiopogonin D' (4) and gracillin (5), respectively.

Key words: Anemarrhena asphodeloides; spirostanol steroidal saponins; UPLC-MS/MS

Objective: To analysis the fragmentation pattern of spirostanol steroidal saponins from Anemarrhena asphodeloides with UPLC-MS/MS.

Results and Discussion: Similar deductive reasoning was applied to spirostane-type RSs 1-5. Two concise and clear [M+NH4]+ and [M+H]+ ions were obviously observed in the ESI+-EPI (CE,8) spectra for RSs 1-5. In the ESI+-EPI (CE,15) spectra, successive losses of sugar moieties one-by-one were also generated to provide [Agly.+H]+ (RSs 1-5). In addition, the fragmentations of aglycone ions were readily observed in the ESI+-EPI (CE, 35) spectra. Two corresponding ion transitions were involved in [S4+H]+→[S4-C8H16O2+H]+→[S4-H2O-C8H16O2+H]+ (Δm= 144 and 18 Da) and [S4+H]+→[S4-H2O+H]+→[S4-H2O-C8H16O2+H]+ (Δm = 18 and 144 Da) for RSs 1-5. This could be explained through the elimination of E rings and water molecules. The major MS/MS fragment pathways of spirostane-type RSs 1-3 were summarized. Whatever spirostanol SSs, all were characterized by preferential loss of a NH3 (17 Da) from an ammoniated precursor ion in the ESI+-EPI (CE, 8) spectra and then C-3 sugar moiety to afford corresponding protonated aglycones in the ESI+-EPI (CE, 15) spectra. As expected in ESI+-EPI (CE, 35) spectra, diagnostic loss of 144 Da from protonated spirostane-type aglycones was attributed to the absence of an oxygen substitution at the F ring while a neutral loss of 160 Da from protonated aglycones could be ascribed to the presence of an OH group (often seen at C-23) in the F ring. The foregoing deduction processes repeated, neutral losses of 142 and 158 Da ions were readily inferred from corresponding protonated spirostanol aglycones for A. asphodeloides SSs. The neutral loss of 142 Da may be explained by the presence of

an additional double bond in the F ring (often seen at Δ25(27)-), while a diagnostic loss of 158 Da could be interpreted by the presence of both a double bond (often seen at Δ25(27)-) and an OH group in the F ring (often seen at C-23).

RESEARCH PROGRESS AND PROSPECT OF MICRORNA IN PREVENTION AND TREATMENT OF OSTEONECROSIS OF THE FEMORAL HEAD

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[Abstract]MicroRNAs (miRNAs) belong to a non-protein coding family of small RNAs and are involved in the physiological and pathological processes of several diseases. They are approximately 22 nucleotides in length and have specific expressions in human tissues or cells. Among these, one group of miRNAs has been confirmed to play fundamental roles in gene regulation in various orthopedics diseases, such as bone tumors, osteoarthritis, and rheumatoid arthritis. The study of miRNAs in the osteonecrosis of the femoral head (ONFH) can improve the understanding of the pathogenesis of the disease. ONFH is an orthopedic disease that is the primary cause of disrupted blood supply to the femoral head and the main symptoms of bone and muscle dysfunction. Recent studies showed that miRNA played a major role in the regulation of the microcirculation of ONFH, damage and repair of blood vessels, local microcirculation dysfunction caused by other diseases, and apoptosis of bone cells. In this study, recent related research results of miRNA and ONFH were analyzed and summarized, and the prospective in the prevention and treatment of the disease was also discussed.

[Keyword]MicroRNA,Osteonecrosis of the femoral head ,Review,Research progress.

1.Introduction Cells contain a variety of non-coding RNAs. Among them, microRNA (miRNA) is considered to widely present in human tissues or cells. In addition, abundant gene regulatory molecules occur in a variety of cell organisms that can affect the output of many protein coding genes. The miRNA gene produces a micro-transcript of approximately 22 nucleotides that acts as an antisense factor for other RNAs [1,2].

Osteonecrosis of the femoral head (ONFH) is a common orthopedics disease, and if not treated in a timely manner, the femoral head would completely collapse in about 80% of the patients, which is rather challenging for the Department of Orthopedics[3].The pathogenesis of this disease includes increased intraosseous pressure, lipid metabolism disorder, intravascular coagulation, damage of microvascular endothelial cells, apoptosis of osteoblasts and osteocytes, and annihilation of the immune system[4]. Previous studies showed that miRNAs can modulate the physiology and pathology of the body through target genes, including cell proliferation, differentiation, apoptosis, and tissue development .

2.Prospects In recent years, miRNA has gradually become the focus of research in bone science. With an increasing number miRNA studies, miRNA has been speculated to have a promising prospect in orthopedic research owing to the specific structure of the femoral head tissues. The detection and analysis of miRNA opened a new research direction for the studies on pathogenesis, diagnostic methods, and treatment approaches of ONFH, thereby postulating the molecular biology and genetic mechanism underlying ONFH. As different pathological factors could lead to differential expressions of different miRNAs, the detection of miRNA could be used to identify the pathogenesis of different ONFH, rendering a targeted treatment and improving the cure rate of patients with ONFH. In addition, the present study proposed that the strategies for prevention and treatment of ONFH could be divided into 2 directions in the future: (1)silencing the highly expressed disease-related genes through miRNA or similar drugs;(2)silencing the highly expressed disease-related miRNA through anti-miRNA molecules. Therefore, miRNA could not only guide doctors in the clinical treatment but also aid in designing an efficient miRNA-targeting drug .

Presently, the studies on the prevention and treatment of ONFH are still at the preliminary stage. A majority of the target genes and regulatory pathways related to ONFH have not yet been elucidated. The application of miRNA technique in the treatment of ONFH is still at the experimental stage, and the precise role of miRNA in the occurrence, development, prognosis, and treatment of ONFH needs further studies. However, owing to the rapid development of miRNA detection technology and the biological characteristics based on the regulation of gene and chromosome level, novel approaches for the prevention and treatment of ONFH would be available in the future.

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