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.

This work was supported by Major State Basic Research Development Program (973Program) of China (2013CB531801).

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