ABSTRACT

Plant species Chinese lantern (*Physalis alkekengi* L.) represents a rich source of various bioactive metabolites, therefore their detailed characterization in different plant parts is crucial for its safe and efficient use. By screening analyses of *P. alkekengi* L. var. *franchetii* cuticular waxes we showed that beside characteristic lipids, phytosterols and triterpenoids, these waxes also contain flavonoids and carotenoids. We developed a non-targeted approach for the separation, isolation and identification of methylated flavonoids present in Chinese lantern cuticular waxes. A rapid and simple separation on HPTLC silica gel was developed for screening of methylated flavonoids. HPLC–UV–MS^n and HPLC–UV methods using a C6-phenyl and a C18 stationary phases were also developed, respectively. For both methods, the right combination of temperature and tetrahydrofuran as a mobile phase modifier was shown to be crucial for the baseline separation of all studied compounds. By applying a semi-preparative chromatography on the C18 column pure analytes were isolated and identified by NMR as four 3-*O*-methylated flavonols: myricetin 3,7,3'-trimethyl ether, quercetin 3,7-dimethyl ether, myricetin 3,7,3',5'-tetramethyl ether and quercetin 3,7,3'-trimethyl ether. The simple and fast isocratic HPLC–UV–MS^n method enables chromatographic fingerprinting of external methylated flavonols, which can be useful in quality control of *P. alkekengi* L. var. *franchetii*. We explained the mechanism of separation of these metabolites by HPLC, which establishes a foundation for future development of chromatographic methods for methylated flavonols and related compounds.

We developed the first HPTLC and HPTLC–MS/MS methods for characterization of structurally similar and complex pharmacologically active physalins from crude extracts of Chinese lantern. Among tested HPTLC adsorbents (silica gel, RP-18, RP-18 W, cyano), silica gel provided the best selectivity, resolution and the shortest developing time. The majority of physalins was detected in absorption-reflectance mode, while some could only be detected in fluorescence-reflectance mode after derivatization with 2.5% sulfuric(VI) acid detection reagent. Using post-chromatographic derivatization we were able to discover an impurity present in the only commercially available standard (physalin L). The impurity could not be detected using HPLC–UV methods. Moreover, by employing (U)HPLC–MS/MS we also confirmed that physalin L and its impurity could not be separated by existing (U)HPLC methods. The impurity was identified by HPTLC–MS/MS method as 2,3,25,27-tetrahydrophysalin A. HPTLC in HPTLC–MS/MS methods provided an alternative selectivity, better sensitivity and higher resolution. High spectral background and ion suppression originating from the use of silica gel plates in combination with an acidic developing solvent were efficiently resolved by two successive plate pre-developments. This significantly improved the sensitivity of HPTLC–MS/MS method. We introduced an innovative simultaneous hyphenation of HPTLC with a triple quadrupole and an ion trap mass analyzer, which enabled a reliable and straightforward non-targeted characterization of physalins and determination of their types. The applicability of developed HPTLC-densitometric and HPTLC–MS/MS methods was demonstrated by the analyses of physalins from the Chinese lantern aqueous extracts, prepared by an optimized fast and simple extraction method under reflux. Differences in physalin profiles of different parts of *P. alkekengi* L. var. *franchetii* harvested at different stages of maturity were observed. Husks are proposed as the most suitable plant part for *P. alkekengi* L. var. *franchetii* quality control, because they exhibited the most obvious MS^2 physalin fingerprints with minimal interferences.

Key words: *Physalis alkekengi* L. var. *franchetii*, physalins, methylated flavonoids, TLC, HPTLC, TLC–MS, HPLC–MS^n, UHPLC, NMR, mass spectrometry, densitometry.