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HPLC determination of quercetin using relative molar sensitivity to methylparaben as a single reference

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Abstract

A simple and reliable high-performance liquid chromatography (HPLC) method for the determination of quercetin (QR) was developed. The method requires no QR reference; instead, it uses relative molar sensitivity (RMS) to a single reference, methylparaben (MPB). The RMS of QR/MPB was determined using an offline combination of ^{1}H-quantitative NMR (^{1}H-qNMR) and HPLC/UV or HPLC/photodiode array (PDA). In this study, the RMS represents the sensitivity ratio of QR to MPB per mole under defined HPLC conditions. The QR in two natural food additives was quantified using three different techniques such as ^{1}H-qNMR, UV–Vis spectrophotometry, and our single-reference HPLC method. Based on certain amounts of QR in two food additive products, the similar results using ^{1}H-qNMR (mass fraction: 96.9% and 96.1%) and single-reference HPLC (95.8% and 95.0%) were exhibited. On the other hand, the results using UV–Vis spectrophotometry (98.5% and 99.2%) without separation of QR and others (kaempferol and isorhamnetin impurities) showed slightly higher amounts than other techniques. In these techniques, the single-reference HPLC is advanced for the simple and accurate quantification of QR in food additive products without native standard.

Keywords : quercetin, relative molar sensitivity, quantitative NMR

I Introduction

Currently, high-performance liquid chromatography (HPLC) quantitation of marker compounds predominates in quality control assessments for natural products, such as crude drugs and food additives. Therefore, analytical standards for each analyte need to be commercially available. In most cases, reagent grade is used as an analytical standard, and its purity is regarded as 100%. However, ^{1}H-quantitative NMR (^{1}H-qNMR) has revealed that the absolute purity of these reagents, especially reagents from a natural resource, differ between reagent companies.[1-4] Therefore, the use of certified reference materials (CRMs) that have absolute purity is highly recommended; however, these materials are globally scarce. To resolve the unavailability of CRMs for each analyte, a single-reference HPLC method employing relative molar sensitivity (RMS) represents a promising tool.

RMS is defined as the sensitivity ratio of an analyte to a single reference per material amount (unit mole) under defined HPLC conditions. The RMS can be determined via an offline combination of ^{1}H-qNMR and HPLC (Fig. 1); therefore, the value is more reliable than those traditionally determined using only chromatography without considering the absolute purities of the analyte and the single reference. Even if there is no CRM of the analyte, an HPLC method using a single reference as a calibrant can be developed using an RMS normalizing detector response.[5-18] The single-reference HPLC can contribute to improve the reliability of analytical results which are important requirement for maintaining public health and safety in the field of food chemistry.[8-14, 16, 17]

Quercetin (QR), a plant flavonol, is a well-known phytochemical (Fig. 2). QR can be obtained from plants as glycosides; these glycosides then undergo hydrolysis and subsequent purification to release aglycones. QR has strong antioxidative activity, and QUERCETIN, a substance obtained from a natural resource, has been used as a

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