Ultra-fast LC analysis of soy isoflavones in foods and dietary supplements using newly developed 2 μm RP-column designed for polar compounds

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1. Introduction

Soybeans are known to contain 9 kinds of isoflavone glycosides and their corresponding aglycones (Fig.1). Soybeans are known to contain 9 kinds of isoflavone glycosides and their corresponding aglycones (Fig.1). Soybeans are known to contain 9 kinds of isoflavone glycosides and their corresponding aglycones (Fig.1). Soybeans are known to contain 9 kinds of isoflavone glycosides and their corresponding aglycones (Fig.1). Soybeans are known to contain 9 kinds of isoflavone glycosides and their corresponding aglycones (Fig.1). Soybeans are known to contain 9 kinds of isoflavone glycosides and their corresponding aglycones (Fig.1).

Optimization of flow rate

The gradient elution of water and acetic acid containing acetic acid has been commonly used in RP-HPLC separation of soy isoflavones. In Fig.2, the influence of acetic acid concentration on soy isoflavone separation is evaluated under equivalent gradient condition. The gradient condition for the resolution of peak 10 and 11 is improved, while the resolution of peak 7 and peak 8 is worse than initial. Considering both resolutions, the mobile phase containing 3% acetic acid would be suitable for this separation.

Figure 3 shows the optimization process of soy isoflavone separation for conventional LC using HydroSphere C18 column. As shown in Chromatograms a and b, changing column length from 150 mm to 50 mm and changing flow rate from 1.0 ml/min to 1.5 ml/min with 3 μm particles reduce the analysis time without losing resolution. Furthermore, the resolution of a critical pair (peak 10 and 11) is improved by optimization of gradient slope and 12 isoflavones are resolved completely within 10 min as shown in Chromatogram c. Figure 4 shows the method transfer from conventional LC to ultra-fast LC using the newly developed 2 μm particle YMC-UltraHT HydroSphere C18. The 2 μm HydroSphere C18 has the equivalent separation selectivity to that of 3 μm and 5 μm particles, so it can be easily scaled down without changing elution conditions, as shown in Chromatograms a and b.

2. Experiments

The gradient elution of water and acetic acid containing acetic acid has been commonly used in RP-HPLC separation of soy isoflavones. In Fig.2, the influence of acetic acid concentration on soy isoflavone separation is evaluated under equivalent gradient condition. The gradient condition for the resolution of peak 10 and 11 is improved, while the resolution of peak 7 and peak 8 is worse than initial. Considering both resolutions, the mobile phase containing 3% acetic acid would be suitable for this separation.

Figure 3: Optimization of conventional LC conditions with 3 μm particles

Figure 4: Method transfer from conventional LC with 3 μm to ultra-fast LC with 2 μm

3. Application

The ultra-fast LC method using 2 μm YMC-UltraHT HydroSphere C18 was applied to the determination of isoflavones in various soy foods and dietary supplements. Major components in soy foods (soybeans or tofu) were malonylated derivatives.

Figure 5: Comparison of soybean isoflavone separation among 2 μm HydroSphere C18 and commercially available sub-2 μm

Figure 6: Analysis of extracts obtained from various soy foods and dietary supplements

4. Conclusions

The effective analysis methods of soy isoflavones for both conventional LC and ultra-fast LC were developed using HydroSphere C18 column which specially designed for separation of polar compounds. HydroSphere C18 showed superior selectivity and resolution for polar isoflavones.

In optimized method for conventional LC using 50 x 4.6 mm column packed with 3 μm particles, 12 isoflavones were separated completely within 10 min.

The conventional LC method was easily transferred to ultra-fast LC method using 50 x 20 mm column packed with 2 μm particles without changing elution conditions and losing resolution. This ultra-fast LC method within 3.3 min was applicable to determination of isoflavones in various foods.

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References