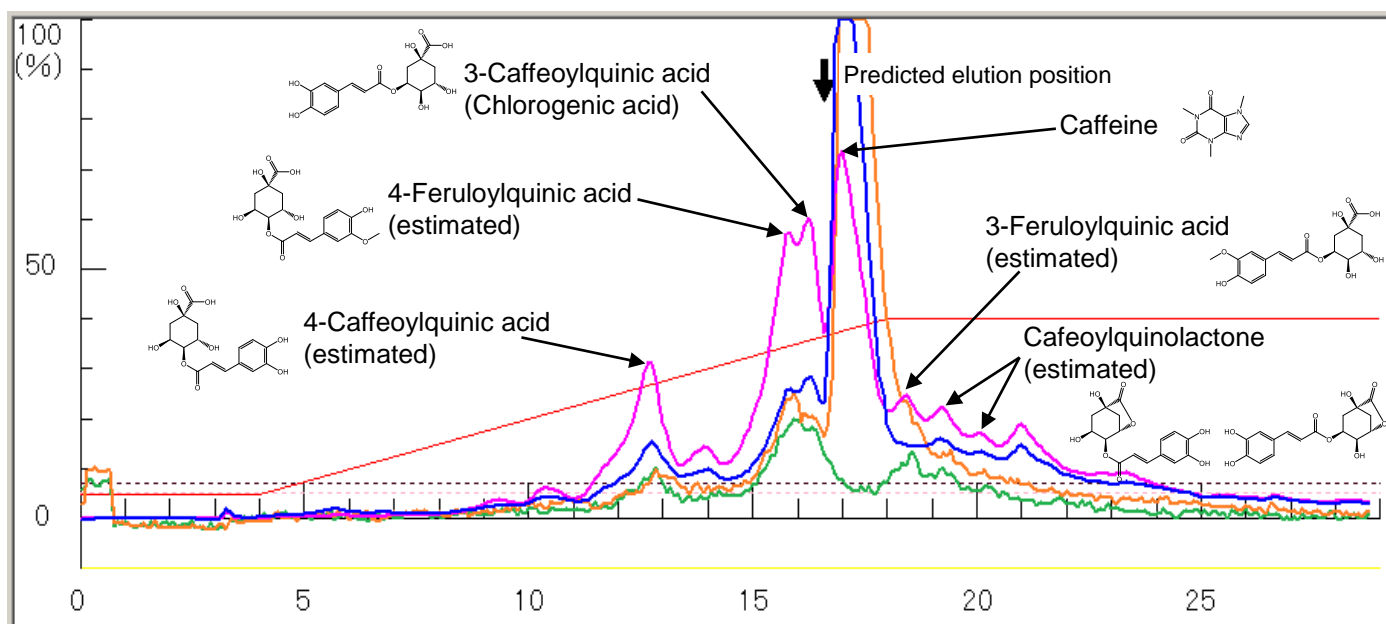


Sample Separation with High Resolution 30 μ m ODS Column Chromatography of Chlorogenic acids and Caffeine in Coffee

There are many cases where extracts from natural products are mixed with compounds having similar structures. In such a case, since the physical properties of each compound are similar, separation by chromatography becomes very difficult.

For example, isolating chlorogenic acids and caffeine in coffee; Chlorogenic acids are a generic name for dehydration condensates of caffeic acid, its derivatives and quinic acid, which are known to be complex compounds including structural isomers with different binding sites of caffeic acid derivatives and quinic acid, analogous substances and their lactones.



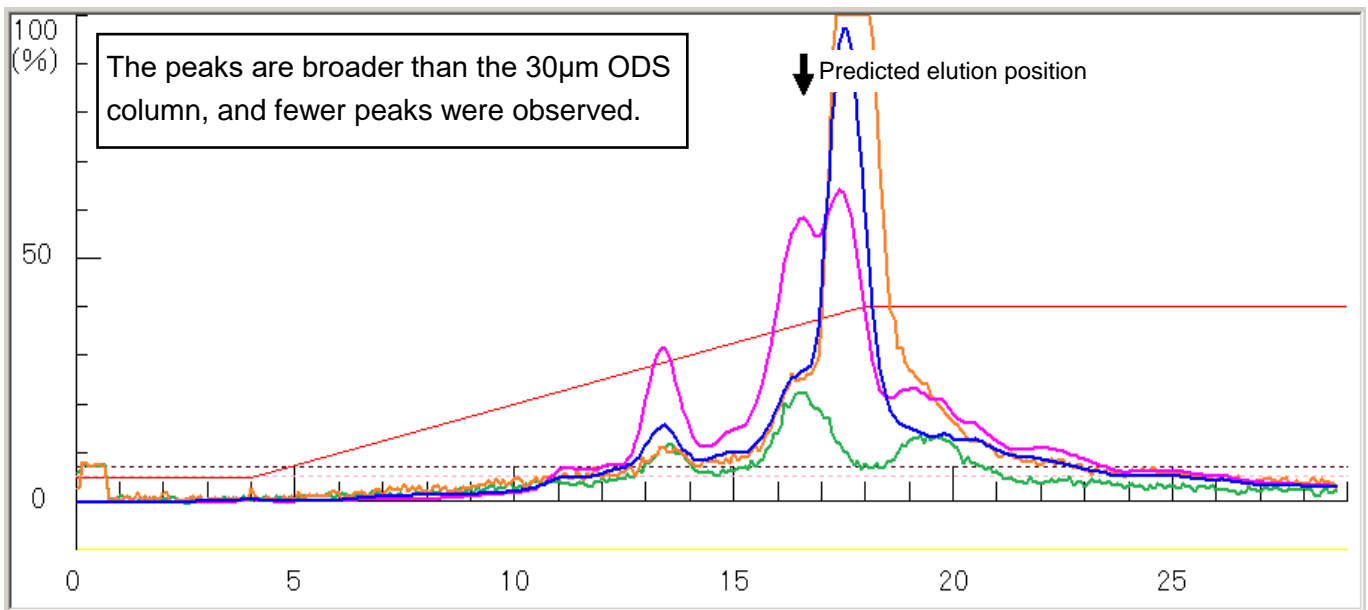
Sample: 5ml of 0.5% formic acid in 25ml
of regular coffee
Column: Universal Premium ODS, L-size, 40g
Gradient: 5% → 40% MeOH

Detector: — UV (254nm)
— UV (280nm)
— MS (TIC positive)
— MS (TIC negative)

It was confirmed that chlorogenic acids were eluted as many peaks before and after caffeine by performing chromatography using a 30 μ m ODS column. In addition, 3-caffeoylquinic acid (chlorogenic acid) and caffeine were confirmed by MS spectrum, and peaks of 4-caffeoylquinic acid (estimated), 3-feruloylquinic acid (estimated) and caffeoylquinolactone (estimated) were recognized. Since the 30 μ m ODS column has higher resolution than the 50 μ m column, it is very useful for separation of natural products with many mixed compounds.

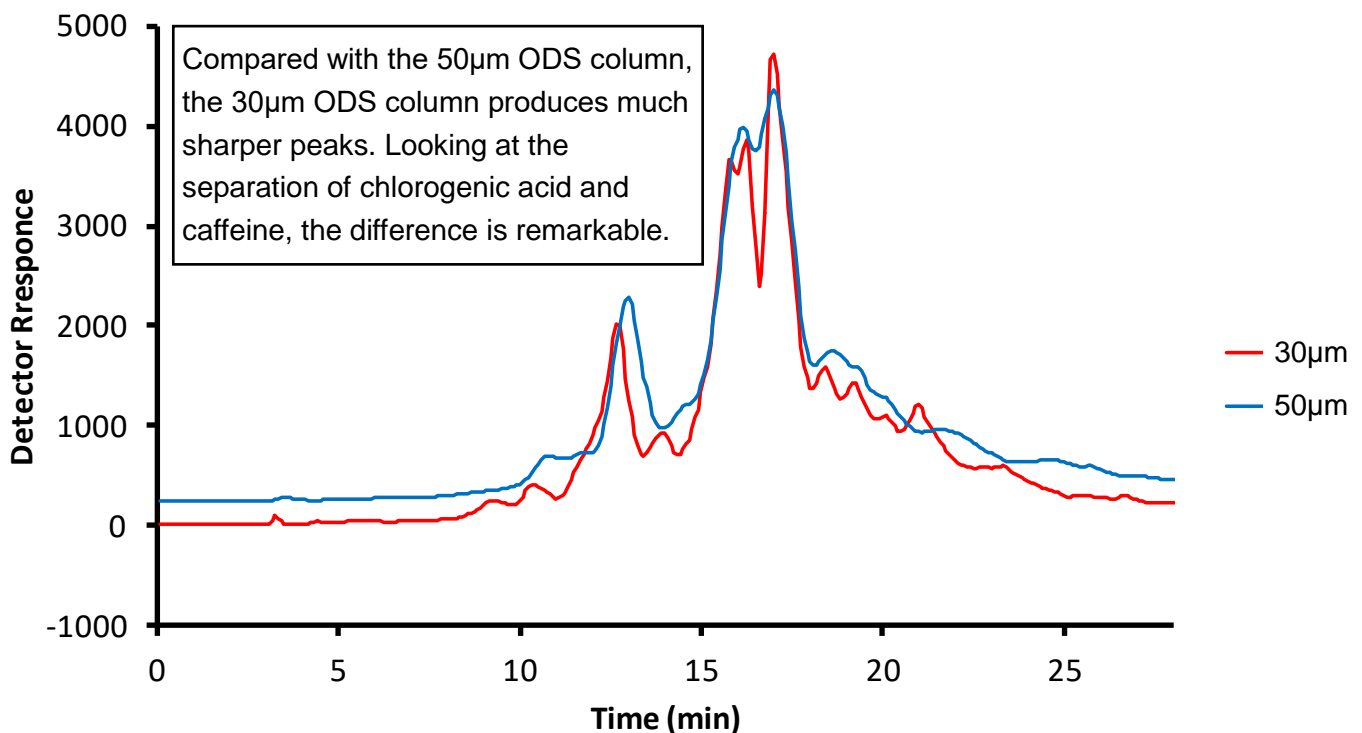
Comparison with 50 μ m ODS columns

■ Chromatogram with 50 μ m ODS column



■ Superposition of chromatograms with UV 280nm

(Move the chromatogram run by a 50 μ m column in the time axis direction so that the peaks of chlorogenic acid and caffeine overlap.)



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