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Asia Pacific

Application Notes
High Performance Liquid Chromatography

Rapid Analysis of Theobromine and Caffeine in Cocoa Powders and Soft Drinks by Ultra Fast Liquid Chromatography (UFLC)

Fast LC analysis, which is essentially based on utilizing sub- or near-2µm particle size column and the state-of-the-art hardware technologies, has attracted attentions in HPLC applications for the impressive high speed. The Shimadzu Prominence UFLC fast LC system with the Shim-pack XR-ODS (C18) column (2.2 µm) offers a choice for extremely fast analysis of samples with excellent resolution, sensitivity and reproducibility[1].

In this Application News, we report a fast LC method for rapid analysis of theobromine and caffeine in cocoa powders and soft drinks using UFLC. Theobromine and caffeine are naturally occurred purine alkaloids, which are known acting as stimulants to the central nervous system and having physiological effects on some biological systems of human. They are found widely in a variety of foods, beverages and pharmaceutical products such as coffee, tea, cola, cocoa and chocolate etc. Analysis of them in these products using conventional HPLC methods has been established in government control labs and manufacturers for the concerns of public health[2]. A fast LC method means a significant enhancement in sample throughput (5~10 times) and productivity compared to a conventional HPLC method. In addition, solvent consumption per sample is reduced dramatically and thus saving cost on organic solvents and the treatment of waste solvents for an analytical lab.

Fast LC method and conditions

A Shim-pack XR-ODS fast LC column of 3.0 x 50 mm and 2.2µm particle size was used in this analysis work. A gradient elution program was employed to run analysis at a total flow rate of 1.2 ml/min (see Figure 1). A complete analytical cycle from one injection to the next injection in a sequence run was 83 seconds, consisting of 10 sec for injection, 43 sec for gradient elution, and 30 sec for column re-equilibration. The data sampling rate of the PDA detector was 40 Hz. The peak width of the compounds studied was about 0.54 sec at 50% peak height and 1.2 sec at 5% peak height. This means that a peak is consisted of at least 50 data points. The injection volume of mixed standard solution was 10µL, and could be increased up to 50µL for standard and extract samples without peak distortion.

The chromatograms of mixed standard solutions of various concentrations are shown in Figure 1. Theobromine and caffeine peaks were found at retention times of 0.674 min and 0.733 min, respectively. The peaks of both compounds are narrow, smooth and well separated under the conditions. Figure 2 shows the calibration curves of theobromine and caffeine at low concentration ranges, respectively. Excellent linearity with R^2>0.9998 was obtained for both compounds within their respect testing ranges: 1.0~100 µg/ml for theobromine and 0.1~60 µg/ml for caffeine. The limit of detection (LOD) was estimated from the result of the lowest concentration sample to be 0.017 µg/ml and 0.020 µg/ml for theobromine and caffeine, respectively.

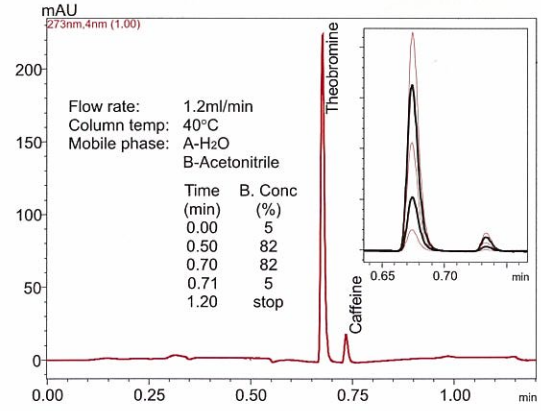

Fig 1. A Chromatogram of mixed standard solutions of different concentrations (Theobromine: 1~10 µg/ml; Caffeine: 0.1~1 µg/ml).

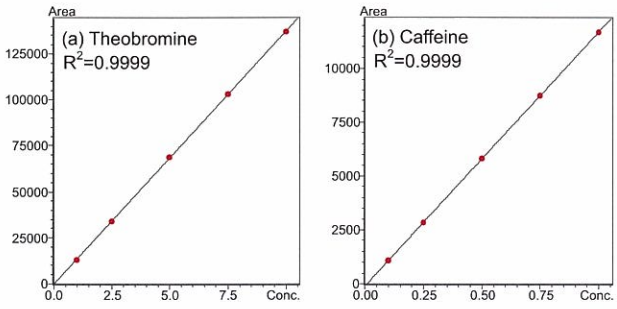

Fig 2. Calibration curves of theobromine(a) and caffeine(b)

Repeatability of a fast analysis method under a fast gradient elution condition is a critical parameter in performance evaluation of the system and column. Table 1. shows the results of five consecutive injections of the same mixed standard solution (theobromine: 25 µg/ml, caffeine: 2.5 µg/ml) and the repeatability of retention time and peak area values. The RSD values of retention time and peak area were lower than 0.036% and 0.074%, respectively. This excellent repeatability is attributed to the superior design and manufacturing of the delivery pumps, flow mixer, auto-sampler, column oven and detector as well as the small internal diameter piping of the whole system. In addition, the relatively low backpressure of the XR-ODS column (< 220 kgf/cm^2 in the current method) under a high flow rate can avoid the possible uneven temperature distribution inside the column. Extremely high backpressure (> 500 kgf/cm^2) created by sub -2 µm particle size column may generate a great amount of heat due to the high resistance when a mobile phase passes through it. An uneven temperature gradient and distribution inside the column may occur and results in a poor repeatability, especially under a fast gradient elution condition.

Table 1 Repeatability of retention and peak area

No. of injection	Theobromine		Caffeine	
	RT (min)	Area	RT (min)	Area
1	0.674	334974	0.733	29161
2	0.674	334424	0.733	29212
3	0.674	335000	0.733	29175
4	0.674	334693	0.733	29187
5	0.674	334961	0.733	29166
mean	0.674	334811	0.733	29180
%RSD	**0.036**	**0.074**	**0.033**	**0.069**

Determination of theobromine and caffeine in cocoa powders and soft drinks

The above fast LC method was used in analysis of extract samples from cocoa powders and some soft drinks available in local supermarket.

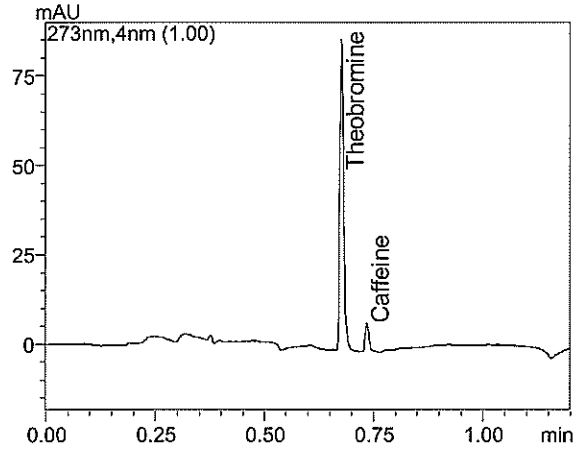

Fig 3. Full chromatogram of cocoa power-1 extract sample

For soft drinks, the aqueous samples were injected to the UFLC directly after dilution and filtration. For cocoa powders sample, the sample was defatted and deproteinized, and then extracted by water at 100°C. The extract samples were diluted and filtered prior to injection.

Figure 3 shows the chromatogram of cocoa power-1 sample. The contents of theobromine and caffeine in this extract solution were 3.90 and 0.44 µg/ml, which were in turn the amounts of 1940 mg/kg and 220 mg/kg, respectively. The quantitative results of the four samples are shown in Table 2.

Table 2 UFLC results of theobromine and caffeine in food samples

Sample	Theobromine	Caffeine	Total
Soft drink C (mg/L)	Not found	92	92
Soft drink P (mg/L)	Not found	102	102
Cocoa powder-1 (mg/kg)	1940	220	2160
Cocoa powder-2 (mg/kg)	1470	150	1620

Summary

The Prominence UFLC with XR-ODS column has demonstrated the superior capability in rapid analysis of therobromine and caffeine without compromise in basic HPLC performance like sensitivity, repeatability and separation resolution. The fast analysis method with an analytical cycle time of 83 seconds allows obtaining results almost immediately after injection and as a result achieving high productivity for QC labs of food, beverage and pharmaceutical industries.

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Reference

1. LC World Talk, p3-7, Winter 2007/International Edition, Shimadzu, 2007.
2. Jaspreet K.C. Ahuja, Betty P. Perloff, *Family Economics and Nutrition Review*, Spring, 2001.

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