Convenient fractionation of polyphenol ingredient from green tea

EP-001

【Keyword】 green tea　polyphenol　fractionation　Conveni-Prep

Introduction

- Polyphenols have a wide variety of effects such as antioxidant, anti-fatigue, and deodorizing .
- Solid phase extraction is often used to obtain the polyphenol fractions when discovering these active ingredient or evaluating the activities from foods.
- By using a fractionation and refining system "Conveni-Prep" with VVC method* ,which is patented technology , it was possible to obtain a polyphenol fraction of tens of mg easily and quickly.

＊Vacuum Vortex Concentration : VVC, Fig.2

Feature of Conveni-Prep

- A fractionation system using the patented VVC method technology; possible to concentrate and dry the fractionated sample in one device.
- As a 60 mL reservoir is used for the sample introduction, almost 50 mL of sample solution can be processed at one time.
- Discharging by a vacuum pump make the fraction works easier even with a large volume reservoir.
- Adsorbent is not prefilled and can be filled only the necessary amount at the time of use; fractionation cost can be saved.
- As the sample and the adsorbent can be stirred at the time of sample adsorption, the recovery rate of the sample with a slow adsorption rate can be improved.

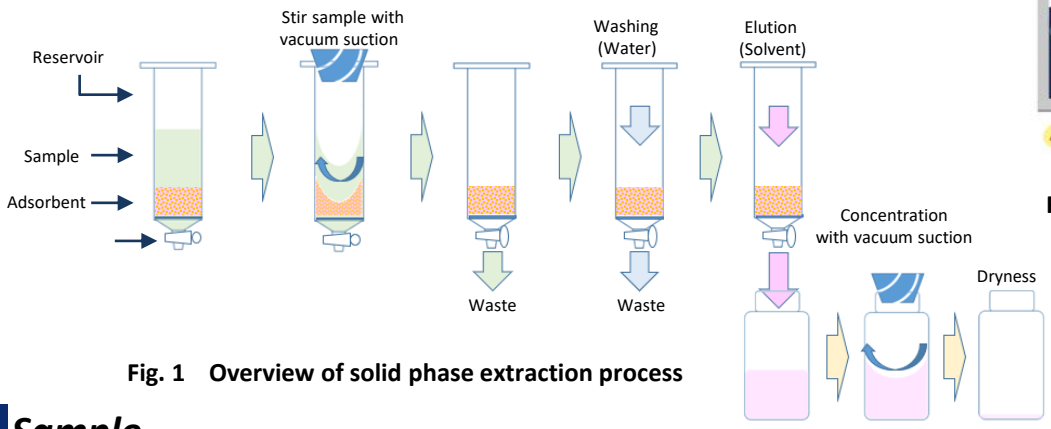

Fig. 1　Overview of solid phase extraction process

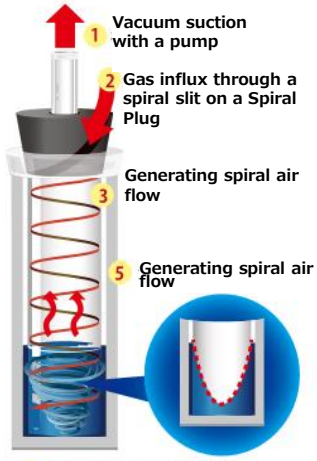

Fig. 2 Mechanism of VVC method

Sample

・Green tea in a plastic bottle

Experiment

<Basic operation>

- Add 10 g of aromatic synthetic adsorbent to the reservoir and perform conditioning with methanol.
- After adding 20 mL of sample to the reservoir, discharge the sample.
- Add 20 mL of water to the reservoir and wash the adsorbent.
- Add 15 mL of methanol to the reservoir and extract the polyphenol fraction (receive in a vial).
- Concentrate vortex by suction while maintaining the extract solution in the vial at 50 ° C.

* The fraction extracted from green tea by the above operation was designated as "polyphenol fraction".

<Evaluations>

① Linearity of the adsorpted amount of polyphenol fraction

In order to evaluate the adsorbance ability of the adsorbent, the amount of the polyphenol fraction when the sample amounts are 10 to 40 mL were confirmed .

② Repetitive use evaluation of adsorbent

The polyphenol fraction amount was confirmed when the adsorbent was repeatedly used.

Fig. 3　60 mL reservoir (Adsorbent 10 g filled)

Conveni-Prep M2

BioChromato

Mining ideas, Aiding laboratories

Web ：https://biochromato.com/
Email：oversea@bicr.co.jp

Results

① Linearity of the adsorped amount of polyphenol fraction

The weight of the polyphenol fraction after evaporation to dryness was measured when the sample volume was 10 to 40 mL.

As shown in Tab. 1 and Fig. 4, there was a fine correlation between the sample amount and the obtained polyphenol fraction amount. It was confirmed that the adsorb ability of this adsorbent is good enough to be used for the quantitative solid phase extraction work. Also, when 20 mL of tea with a high catechin content was treated, more than 100 mg the polyphenol fraction can be obtained.

Tab. 1 Polyphenol fraction amount corresponding to the sample volume

Sample volume (mL)	10	20	30	40
Amount of polyphenol fraction (mg)	9.8	20.2	30.2	40.6

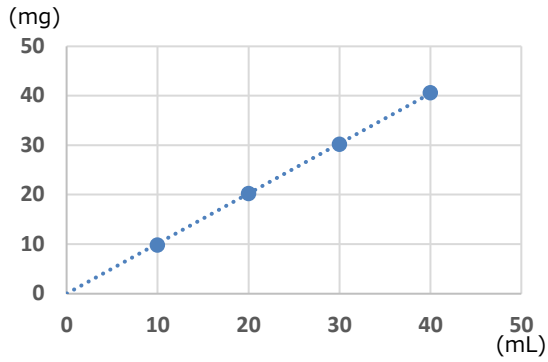

Fig. 4 Correlation between the sample volume and polyphenol fraction amount

② Repetitive use evaluation of adsorbent

Tab. 2 and Fig. 5 show the result when the sample amount was set as 20 mL, and the same adsorbent was repeatedly used. The result of six times repeated use showed relatively fine value, average=19.8 mg and C.V.=3.9%, that meant the adsorbent used in this experiment is sufficiently tolerable with repetitive use.

Tab. 2 Polyphenol fraction amount with 6 times repeated use

Repeated times	1	2	3	4	5	6
Amount of polyphenol fraction (mg)	19.8	18.8	19.0	20.8	20.1	20.3

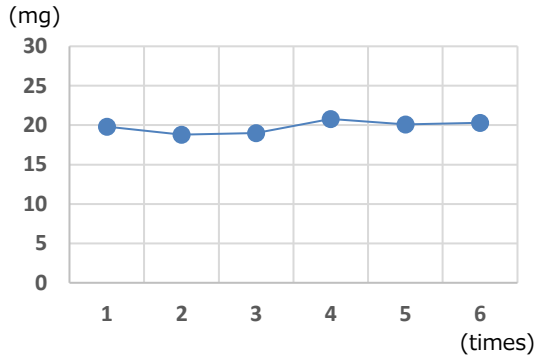

Fig. 5 Polyphenol fraction amount with 6 times repeated use

Fig. 6 shows the HPLC charts of the analysis of polyphenol fractions obtained in first and sixth experiments. It was confirmed that almost same components were obtained as the phenol molecules obtained by the repeated experiments.

HPLC conditions
Column：Inertsil ODS-4 (3.0×100mm)
Mobile phase：A: 50mmol/L NaH2PO4 (pH4)
B:CH3CN
Gradient ：Time(min) 0 ⇒ 15
B (%) 10 ⇒ 45
Flow rate ：0.3mL/min
Column temperature：40℃
Detection wavelength：270nm

Fig. 6 HPLC chromatograms of the polyphenol fractions

Conclusion

・ By using 60 mL reservoir with "Conveni-Prep" , medium volume samples can be processed at once. Since the discharge from the reservoir is carried out mechanically, easy and quick fractionation of polyphenols from green tea is possible.

Also as the evaporation solidification work following to the extraction can be done quickly and easily, this instrument is hoped to be applicated for such works as fractionations of the other active ingredients.

BioChromato
Mining ideas, Aiding laboratories

Conveni-Prep M2

Web ：https://biochromato.com/
Email：oversea@bicr.co.jp