

Note

Rapid determination of caffeine in green tea by gas–liquid chromatography with nitrogen–phosphorus-selective detection

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Caffeine is an important component of tea, comprising 3–5% of the total weight. Many methods for determining caffeine in tea have been reported, including high-performance liquid chromatography^{1–4}, spectrophotometry^{5,6} and gas–liquid chromatography (GLC)^{7,8}. In these methods, a tea sample is extracted with boiling water and the extract is cleaned up prior to the determination. The clean-up procedure usually involves such operations as partitioning (several times), concentration, acid–base treatment and precipitation. This paper describes a simpler and quicker method, based on the sensitive response of a nitrogen–phosphorus detector to caffeine and the difference in solubility between caffeine and other polar compounds in green tea.

EXPERIMENTAL

Materials and apparatus

Analytical-reagent grade toluene, anhydrous sodium sulphate, 4.5 *M* sulphuric acid, 0.01 *M* hydrochloric acid and saturated basic lead acetate solution were used.

A Hewlett-Packard HP-5790A gas–liquid chromatograph equipped with a nitrogen–phosphorus-selective detector and a glass column (1.2 m × 2 mm I.D.) packed with 6% OV-101 on Chromosorb W (80–100 mesh) was used, and also a Shimadzu UV-265 FW spectrophotometer.

Proposed GLC method

Ground tea (1.000 g) was extracted with boiling water (200 ml) for 20 min in a 250-ml erlenmeyer flask. The extract was filtered and an aliquot (0.1 ml) of the filtrate was transferred to a 10-ml volumetric flask. Toluene (9.9 ml) and anhydrous sodium sulphate (*ca.* 1 g) were added. The flask was shaken vigorously for 0.5 min prior to GLC determination.

The operating conditions of the instrument were as follows: oven temperature, 205°C; injector temperature, 230°C; detector temperature, 240°C; carrier gas (nitrogen) flow-rate, 50 ml/min; hydrogen flow-rate, 1.5 ml/min; air flow-rate, 80 ml/min; injection volume, 5 μ l.

Under the above conditions, the retention time of caffeine was 1.9 min and the

detection limit was 0.04%. Quantification was carried out by comparing the peak heights of a standard and the samples.

National standard method

To assess the GLC method, the national standard method was used as a reference. This method was developed by the Tea Research Institute, Chinese Academy of Agricultural Sciences, and was recently approved as a national standard method by the National Standard Bureau (China).

Ground tea (3.000 g) was extracted with boiling water (450 ml) for 45 min and the extract was filtered. An aliquot of the filtrate (20 ml) was mixed with 0.01 *M* hydrochloric acid (10 ml) and saturated basic lead acetate solution (2 ml); the mixture was then diluted to 250 ml with water. The flask was shaken vigorously for 1 min and was allowed to stand for 10 min, after which the solution was filtered. An aliquot of the filtrate (50 ml) was mixed with 4.5 *M* sulphuric acid solution (0.2 ml) in a 100-ml volumetric flask and diluted to 100 ml with water. The flask was shaken vigorously for 1 min, then the solution was filtered. The filtrate was analysed using a UV spectrophotometer at 274 nm.

RESULTS AND DISCUSSION

Selection of extraction time

Tea samples were extracted with boiling water for 10, 20, 30 and 45 min and the caffeine content was determined by the GLC method and by the standard method. The results are given in Table I.

The results suggest that the efficiency of the 10-min extraction is as good as that of a 45-min extraction. Therefore, 20-min extraction was adopted.

Application of the method

Five tea samples were analysed by the GLC method with four replicates for each sample. The coefficients of variation were 1.20, 0.95, 1.42, 0.90 and 3.18%. Three tea samples were analysed by the standard method, with four replicates for each

TABLE I
INFLUENCE OF EXTRACTION TIME ON THE EXTRACTION EFFICIENCY

<i>Sample</i>	<i>Method</i>	<i>Extraction time (min)</i>	<i>Caffeine content^a (%)</i>
A	GLC	10	2.19 ± 0.026
	GLC	20	2.20 ± 0.021
	GLC	45	2.16 ± 0.031
	Standard	20	2.18 ± 0.061
	Standard	45	2.18 ± 0.099
B	GLC	30	3.23 ± 0.029
	GLC	45	3.37 ± 0.107
	Standard	30	3.32 ± 0.212
	Standard	45	3.36 ± 0.086

^a Mean of four replicates ± standard deviation.

sample, and an average coefficient of variation of 3.67% was obtained. The data in Table I suggest that the results obtained by the GLC method are the same as those obtained by the standard method.

An advantage of the GLC method is its simplicity, which not only saves considerable amounts of reagents and time, but also reduces the possibility of errors.

Prior to GLC analysis, sample clean-up procedures are usually needed to remove impurities which may affect accurate quantification. This simplified GLC method is based on the selective response of the instrument to caffeine and the difference in solubility of caffeine and the coextractives. Tea contains about 30% of water-extractable components, mainly polyphenols (80%) and caffeine (10%). When caffeine is injected into a gas chromatograph in the nanogram range, the amount of impurities introduced into the instrument should be in the same range, even if no clean-up treatment is used. Polyphenols are very soluble in water and almost insoluble in toluene. Therefore, when toluene is used to extract caffeine from water, the percentage of polyphenols and other polar impurities entering the toluene phase should be much less than that of caffeine. During analysis with this GLC method, the baseline and the response remained steady after over 100 injections, and no other peaks were observed in the chromatogram, suggesting that the samples were sufficiently clean.

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