

The Determination of Caffeine in Cola Drinks

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A gas chromatographic method for determining caffeine in cola beverages is described. Results are given which show that the designation "Cola Drink" is being applied to two types of soft drink; one type containing 70–200 mg per litre caffeine the other containing little or no caffeine. These two types should be distinguished.

The usual method for determining the caffeine content of cola drinks involves extracting the caffeine from alkaline solution with chloroform and measuring the extinction of the extract at 273 nm¹. This method may lead to high results due to the solvent extracting substances, other than caffeine, having significant extinction at 273 nm (see Table II below). The following method seeks to overcome this difficulty.

Apparatus

1. Pye Gas Chromatograph Series 104 Model 24 (or equivalent instrument).
2. Quickfit stoppered tubes 150 mm × 23 mm.

Reagents

1. *Dichloromethane*: (Redistilled).
2. *Concentrated Ammonia solution*: Sp.Gr. 0.880.
3. *Standard Solution of Caffeine*: A solution of caffeine (B.P.) in dichloromethane (1 ml equivalent to 1.00 mg of caffeine).
4. *Procaine hydrochloride* (B.P.).
5. *Acetone*.
6. *Internal standard solution*: Dissolve 0.12 g of procaine hydrochloride in 50 ml of distilled water, add 20.0 ml of acetone and dilute with water to 100 ml.

Gas Chromatography Conditions

Column: Glass, 5 ft × $\frac{1}{4}$ in.

Support: Phasesep N. AW-DMCS (60–85 mesh).

Liquid Phase: 2 per cent. Versamid 930.

Oven Temperature: 200°C (isothermal).

Detector: Flame Ionisation.

Carrier gas: Nitrogen; 76 ml per minute.

Hydrogen: 76 ml per minute.

Air: 550 ml per minute.

Attenuation: 200 (equivalent to 2×10^{-10} amps full scale).

Chart Speed: 2.5 mm per minute.

Retention Times: Caffeine 4.0 minute.

Procaine 7.2 minute.

Method

Pipette 50.0 ml of the de-gassed cola drink into a 100-ml separating funnel, add sufficient concentrated ammonia solution to ensure that the liquid is alkaline (5–6 drops usually suffice), then 10 ml of dichloromethane and shake gently to extract the caffeine. Allow to stand and separate; run off the lower organic layer through a small funnel, containing a cotton wool plug previously washed with dichloromethane, into a tube fitted with a ground glass stopper. Carefully evaporate the solvent by means of a hot water bath. Repeat this procedure with five further 10-ml portions of dichloromethane. When the solvent has been removed from the sixth extract, note the amount of residue in the tube as follows:—

(a) easily visible white residue

(b) little or no white residue

If (a), measure into the tube by pipette 5.0 ml of the procaine solution.

If (b) measure into the tube by pipette 1.0 ml of the procaine solution. Stopper and shake the tube until the caffeine residue is completely dissolved. This is the Sample Solution.

Prepare a standard caffeine solution by measuring 5.0 ml standard caffeine solution into a similar glass tube, and evaporating the solvent on a hot water bath. When all of the solvent has been removed pipette 5.0 ml of the procaine solution into the tube and shake as before until the caffeine is dissolved. This is the Working Standard.

Inject aliquot portions of 2 microlitres of the Working Standard onto the G.L.C. column. From the trace calculate the ratio

$$S = \frac{\text{Peak height for caffeine}}{\text{Peak height for procaine}}$$

The graph of peak height versus mg caffeine is linear over the range 0–6 mg of caffeine.

Inject 2- μ l aliquots of the Sample Solution onto the G.L.C. column and calculate

$$R = \frac{\text{Peak height for caffeine}}{\text{Peak height for procaine}}$$

Calculations

(a) When 5.0 ml of procaine solution is used,

$$\text{caffeine in sample} = \frac{100 R}{S} \text{ mg per litre.}$$

(b) When 1.0 ml of procaine solution is used,

$$\text{caffeine in sample} = \frac{20 R}{S} \text{ mg per litre.}$$

$$(c) \text{ Generally, caffeine in sample} = \frac{1000 R.V_p}{S.V_c} \text{ mg per litre}$$

Where V_p = Volume of procaine standard used and V_c = Volume of sample solution taken.

Results

The above method has been applied to a number of different "Cola" and "Cola Drink" samples. The results are presented in Table I.

TABLE I
CAFFEINE CONTENT OF COLA AND COLA DRINKS

Sample	Caffeine content <i>mg per litre</i>
A	188
B	132
C	130
D	128
E	123
F	122
G	114
H	94
J	77
K	75
L	70
M	2
N	less than 1
O	less than 1
P	less than 1
Q	less than 1

Certain of these samples were also examined using the method detailed in "The Chemical Analysis of Food"¹. The results are given in Table II.

TABLE II
COMPARATIVE RESULTS FOR CAFFEINE CONTENTS OF COLA PRODUCTS

Sample	Caffeine <i>mg per litre</i> G.L.C. method	u.v. method
F	122	129
H	94	143
L	70	87
N	less than 1	6
P	less than 1	8

Discussion

The method described has been found to give recovery of 95 to 98 per cent. of the caffeine added to cola preparations.

The results tabulated in Table I indicate that the designation "Cola Drink" or "Cola" is being used for two different beverages viz: (a) beverages containing a significant amount of caffeine, in the range 70–200 mg per litre, and (b) beverages containing little or no caffeine.

The major share of the market demand for cola drinks is supplied by the caffeine-containing cola drinks and thus it may be argued that this type of cola drink represents "the substance demanded by the purchaser" under Section 2 of the Food and Drugs Act, 1955.

Further, a certain amount of concern has been expressed in a "Which?" report² regarding the possible effect of caffeine on children who consume a lot of cola drinks. At present a parent buying cola drinks for children has no means of telling whether or not the particular cola drink purchased contains caffeine.

It is contended, therefore, that the designations applied to the two types of cold drink should be such as would enable an intending purchaser to know whether he was buying a cola drink containing caffeine or a similar product which contains little or no caffeine. That such a distinction should be made is supported by the definition of "appropriate designation" given in The Labelling of Food Regulations, 1970 (Regulation 3) which requires that the appropriate designation of a food should be sufficiently specific to indicate to an intending purchaser the true nature of the food.

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References:

1. Pearson, D., "The Chemical Analysis of Foods", Churchill, London, 6th Edition, 1970.
2. Consumers Association, "Which?", London, August, 1971.