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Note

Simple method for the determination of alkaloids in cocoa using paper chromatography and UV spectrometry

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Theobromine and caffeine are the two most abundant alkaloids in cocoa and chocolate. The sum of the alkaloid contents on a fat and moisture-free basis can be used as an accurate indicator of cocoa content[1].
Analyses using high-performance liquid chromatography (HPLC)[1–4] and thin-layer chromatography (TLC)[5] have been published for theobromine and caffeine. This paper reports a simple paper chromatographic method for qualitative and quantitative analysis. The method includes paper chromatographic extraction, which yields an extract suitable for UV spectrophotometry. The method is suitable for routine quality control analyses because only standard laboratory equipment is needed.

EXPERIMENTAL

Materials

Paper strips (Whatman 3 MM or an equivalent thick paper) were cut into strips measuring 5 × 20 cm. Weighing tubes consisted of open-ended glass tubes about 12 cm × 2–3 mm I.D. One end of the tube was narrowed by melting in a flame.

Standards and reagents

Analytical-reagent grade chemicals were used when available. A 10 mg/kg standard solution of caffeine was prepared in water containing 1.0% ammonia. Theobromine standard solution was prepared at a concentration of 10 mg/kg in the same solvent. Other reagents included light petroleum (b.p. 40–60°C) and *n*-butanol saturated with concentrated (25%) ammonia.

Paper chromatography

A sample of about 10 mg of pure cocoa powder, or correspondingly more of a product containing additional ingredients such as sugar or milk, was measured in a weighing tube on an analytical balance. The contents of the tube were streaked in a broad zone across a paper strip, which was kept in a horizontal position, and the emptied tube was weighed again. The material applied to the starting zone was moistened with diluted ammonia solution (12.5%). The strip was then transferred into a chromatographic chamber for ascending chromatography, carefully avoiding any spillage.

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Fats were first removed by chromatographing the paper with light petroleum for about 1.5 h. The paper was then chromatographed with *n*-butanol, saturated with ammonia, for 1.5–2 h. The chromatography was repeated after a short drying period. If necessary, the chromatogram could be left overnight hanging above the solvent in the chamber. Finally, it was re-chromatographed for 3–4 h. The dried chromatogram was observed under UV light (254 nm) to locate the alkaloids and to ensure that theobromine was well separated from the starting zone and that caffeine had not migrated too near to the solvent front. The alkaloids were outlined (Fig. 1).

The marked ˡ ınds were then cut out and eluted for 1 h in stoppered test-tubes containing a measu ⁻1 volume of 5–25 ml of dilute ammonia solution (1%). The tubes were gently sha n occasionally during the elution.

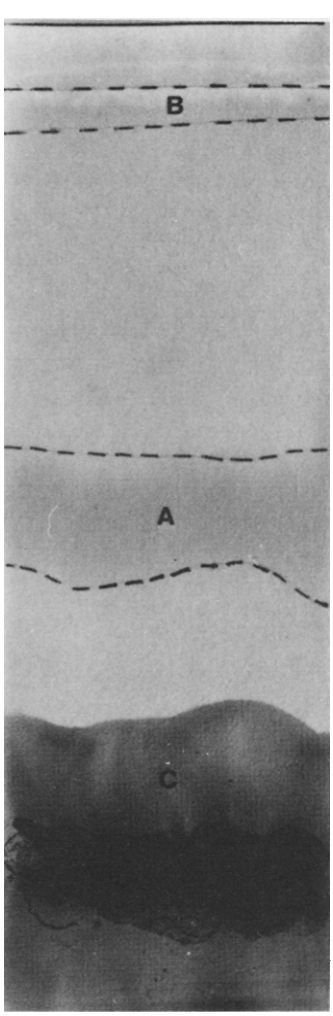

Fig. 1. Chromatogram under UV light (254 nm). A, Theobromine; B, caffeine; C, sample.

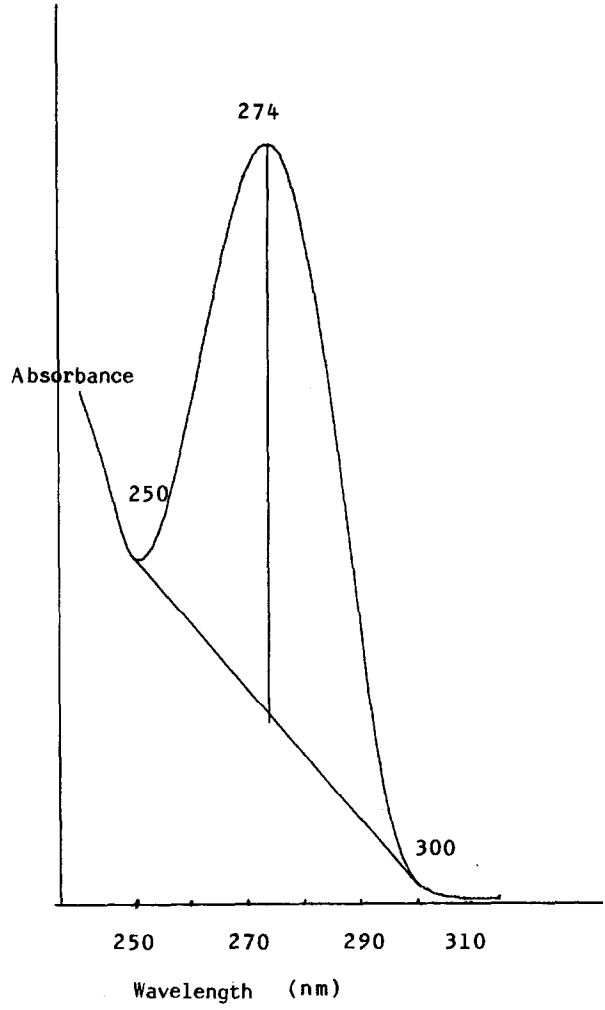

Fig. 2. UV absorption spectrum of theobromine. A tangent was drawn through the points at 250 and 300 nm. The maximum absorption was measured at 274 nm with the tangent as the base.

Spectrophotometric determination

The alkaloids were determined with the aid of their UV absorption spectra. The absorption maxima of theobromine and caffeine were at 274 and 275 nm, respectively, in 1.0% ammonia solution (Fig. 2).

RESULTS AND DISCUSSION

The small amount of sample material used in the analysis necessitated careful homogenization. In an 8-h chromatography, theobromine migrated a good distance from the original zone of application but caffeine was obscured in the solvent front. We found, however, that a single run with a shorter time was not sufficient for theobromine. When both alkaloids were determined together, multiple runs were necessary. A multiple chromatographic mode was employed, including two short runs of about 1.5 h and one longer run of about 4 h. The removal of fats from the chromatogram was effected with either light petroleum or diethyl ether. No losses of caffeine or theobromine were observed using either method, even though according to the literature these purines are more soluble in diethyl ether than in light petroleum.

Several replicate theobromine and caffeine analyses were carried out by the paper chromatographic method using two brands of pure cocoa powder. The new chromatographic method was tested using an HPLC method[1]. The results obtained with the paper chromatographic method compared well with those achieved by HPLC. The results are given in Tables I and II.

TABLE I

DETERMINATION OF THEOBROMINE IN COCOA POWDERS USING PAPER CHROMATOGRAPHY (PC) AND HPLC[3] AS A REFERENCE METHOD

Sample No.	*PC**			*Amount found by HPLC (mg/g)*	*Recovery, PC/HPLC (%)*
	Amount found (mg/g)	*S.D. (mg/g)*	*R.S.D. (%)*		
1	21.99	0.591	2.69	22.56	97
2	20.89	0.411	1.97	21.46	97

* Average results for nine replicate analyses.

TABLE II

DETERMINATION OF CAFFEINE IN COCOA POWDERS USING PAPER CHROMATOGRAPHY (PC) AND HPLC[3] AS A REFERENCE METHOD

Sample No.	*PC**			*Amount found by HPLC (mg/g)*	*Recovery, PC/HPLC (%)*
	Amount found (mg/g)	*S.D. (mg/g)*	*R.S.D. (%)*		
1	0.90	0.069	7.67	0.91	99
2	1.27	0.025	1.99	1.30	98

* Average results for nine replicate analyses.

TABLE III

DETERMINATION OF THEOBROMINE IN DIFFERENT COCOA PRODUCTS BY PAPER CHROMATOGRAPHY (PC) AND HPLC[6] AS A REFERENCE METHOD

Sample No.	*Material*	*Amount found (mg/g)*		*Recovery, PC/HPLC (%)*
		PC	*HPLC*	
1	Cocoa powder	25.3	26.0	97
2		19.4	18.7	104
3		22.5	24.0	94
4		24.0	24.2	99
5		19.0	17.7	107
6		21.7	22.0	99
7		23.4	26.0	90
8		26.8	28.0	96
Mean				98
9	Cocoa powder with sugar	5.4	5.3	102
10		5.4	5.3	102
11		5.7	5.3	107
Mean				104
12	Cocoa powder with sugar and milk	3.2	3.3	97
13		2.6	2.9	90
14		3.4	3.5	97
15		3.2	3.4	94
16		2.6	2.7	96
17		4.0	4.1	98
Mean				95

Analyses of other cocoa products, such as cocoa powders with milk and sugar added or chocolate products, suggested that the method should be applicable to cocoa-containing foods in general (Table III). These results were tested with a modified HPLC method[6] including basic (pH 9) extraction of theobromine.

Caffeine could also be determined in such foods as coffee, tea and cola drinks. Moreover, caffeine alone could be separated rapidly, requiring only a single, short chromatographic run.

CONCLUSION

The main cocoa alkaloids in cocoa powder and cocoa products, theobromine and caffeine, were isolated by paper chromatography and determined by UV spectrophotometry. As a separate extraction step was not needed, the sample was applied directly to the baseline and chromatographed. The results compared well with those obtained using HPLC.

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