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Note

Traditional oriental medicines

I. Black Pearl: identification and chromatographic determination of some undeclared medicinal ingredients

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Black Pearl is a round (10 mm diameter), black herbal pill that is labelled by the manufacturer on Grand Cayman Island as a herbal food supplement containing twenty-two herbs in a honey base. Its physical appearance as well as its smell and the list of ingredients on the label is very close to the traditional Chinese pill, Nan Lien Chui Fong Toukuwan[1]. This study was initiated because, from past experience with Chui Fong[2], the presence of added prescription drugs was suspected. Primary regulatory concern with respect to a product of this nature can be satisfied by the demonstration of the presence of undeclared medicinal ingredients. However, the degree of regulatory response may require the evaluation of the health hazard associated with the product. Accordingly, estimates of the amounts of the medicinal ingredients and the average daily dosage associated with the use of the product may be required.

Although detailed and rigorous thin-layer chromatographic schemes and systems have been described[3,4], the use of the extraction method and thin-layer system used here is particularly applicable to ethnic medicines. From experience these products are formulated often to contain a stereoidal anti-inflammatory, a diuretic and a sedative. The extraction method and thin-layer chromatographic system employed here has been found to be an efficient method for the identification of many of the substances found in ethnic medicines. In combination with direct probe mass spectrometry the identification of a wide variety of substances is facilitated.

The HPLC method was developed specifically for the determination of the added drugs found in Black Pearl pills. Sample preparation involved the crushing of the pill and extraction of the soluble material in solvent. The presence of other material did not interfere with the quantitation of the analyzed drugs by high-performance liquid chromatography (HPLC). The analysis revealed the presence of significant amounts of an anti-inflammatory (indomethacin), an analgesic (mefenamic acid), a tranquillizer (diazepam), and a diuretic (hydrochlorothiazide). Prolonged consumption of products such as Black Pearl containing undeclared amounts of prescription drugs may cause serious adverse health consequences[2].

EXPERIMENTAL

Drugs and chemicals

Ethyl acetate was distilled-in-glass grade (Caledon Labs., Georgetown, Canada). Methanol and acetonitrile were HPLC grade (J. T. Baker, Phillipsburg, NJ, U.S.A.). Monobasic potassium phosphate (J. T. Baker) and orthophosphoric acid (British Drug Houses, Toronto, Canada) were reagent grade. Water was obtained from a Nanopure II system (Barnstead, Boston, MA, U.S.A.). All drugs were obtained from the reference collection of the Health Protection Branch. The Black Pearl pills were obtained through investigative activities of the Health Protection Branch. The labels of all samples received carried no lot numbers but indicated the product originated in the British West Indies.

Thin-layer chromatography

Sample preparation. One pill was powdered and placed in a test tube to which was added about 2 ml of water and 2 ml of ethyl acetate. Dissolution was assisted by stirring with a glass rod. The mixture was centrifuged at approximately 400 *g* for 5 min using a bench-top centrifuge (Fisher Scientific, Ottawa, Canada) and approximately 0.5 ml of the ethyl acetate extract was applied manually to the plate with a disposable pipet.

Plates. Silica gel GHLF 0.25 mm plates (20 × 20 cm) were from Analtech, Newark, DE, U.S.A.

Solvents. System A was ethyl acetate, system B ethyl acetate–methanol (4:1, v/v).

Procedure. The plate was developed first in solvent system A. The bands were identified using short-wave UV light and the substances were extracted from the silica gel using ethyl acetate containing a small amount of methanol. The band corresponding in R_F to hydrochlorothiazide was extracted and the material obtained was redissolved in ethyl acetate and chromatographed using solvent system B.

Identification of substances

A 4610B quadrupole instrument (Finnigan MAT, San José, CA, U.S.A.) operating in electron impact mode was employed. Spectra were obtained by direct probe inlet of the residue obtained from the extraction of the thin-layer plate. Gas chromatographic introduction of the sample residues was precluded due to the wide range of volatility and polarity of the substances. Identification of the drug substances was made by comparison of the mass spectra obtained from the isolated materials with those obtained from authentic standards.

High-performance liquid chromatography

Instruments. The apparatus consisted of a Spectra-Physics (Santa Clara, CA, U.S.A.) SP8100 liquid chromatograph (with integral autosampler), a Spectra-Physics SP8440 UV–VIS detector (operated at 240 nm, because of the applicability of this wavelength to steroid detection) and a Spectra-Physics SP4200 computing integrator.

Conditions. The reversed-phase column which was operated at ambient temperature was an octyl coated silica of 10-μm particle size, 250 × 4.6 mm, RP-8 Spheri-10 (Brownlee Labs, Santa Clara, CA, U.S.A.). The mobile phase was a two component system made from acetonitrile (A) and 0.05 *M* potassium dihydrogenphosphate pH

3.3 (B). The pH of the buffer was adjusted by the addition of concentrated phosphoric acid. The solvent gradient program was as follows, at a flow-rate of 1 ml/min:

Time (min)	*%A*	*%B*
0.00	15.0	85.0
10.00	15.0	85.0
12.00	50.0	50.0
40.00	50.0	50.0
50.00	70.0	30.0

Stock solutions. Stock solutions of indomethacin (1.33 mg/ml), mefenamic acid (1.63 mg/ml), diazepam (0.073 mg/ml) and hydrochlorothiazide (0.6 mg/ml) were prepared in methanol.

Standard solutions. Five solutions were prepared to contain the four drugs over the concentration ranges shown in Table I. The concentrations were based on preliminary chromatographic investigations.

Mixed standard. A mixed standard solution was prepared in methanol to contain each of the substances at one tenth of the concentration in the stock solution.

Sample preparation. Single pills were crushed in a pill crusher and transferred to a vial. Composite was weighed directly into the vial. The contents of the vial were then sequentially extracted using an ultrasonicator (10 min) with four 10-ml portions of methanol. The methanol extracts were combined in a 50-ml volumetric flask and the volume completed with methanol. About 2 ml of the solution was filtered through cotton and 10 μl injected into the HPLC system using the autosampler.

RESULTS AND DISCUSSION

Initial chromatography of the tablet extracts in solvent system A indicated the presence of three bands corresponding in R_F to indomethacin, diazepam and hydrochlorothiazide. No other significant bands were detectable. As it was suspected that the band corresponding to the last substance was impure, the extracted material was re-chromatographed in solvent system B. Two distinct bands were then detected corresponding in R_F to hydrochlorothiazide and mefenamic acid. The R_F of the reference standard drugs on the two TLC systems are shown in Table I.

The retention times and relative responses of the same substances by HPLC are also shown in Table I. The linearity of the system was verified by injection of standard solutions. The detection of the four drugs was found to be linear over the required concentrations. A summary of the data and the minimum detectability of each drug is shown in Table I.

The extraction efficiency of the procedure was verified by analyzing the content of each of the drugs in unpooled sequential 10-ml methanol extracts. The results are shown in Table II. Greater than 95% of each of the drugs was extracted in the first and second extracts. The recovery of the method was determined by adding, to two weighed aliquots of a homogeneous composite, aliquots of a methanolic solution containing each of the four drugs. The amounts added corresponded to approximately 35 and 50% of the amount of each of the drugs originally found to be present.

TABLE I

TLC AND HPLC PROPERTIES OF REFERENCE SUBSTANCES

Drug	*TLC*		*HPLC*				
	R_F		t_R *(min)*	*Relative response*	*Minimum detectability (mg/tablet)*	*Linearity*	
	Solvent A	*Solvent B*				*Concentration (mg/ml)*	*Correlation coefficient*
Hydrochlorothiazide	0.42	0.64	7.76	0.09	0.5	0.0365–0.170	0.990
Diazepam	0.50	0.68	27.37	1.00	0.1	0.00515–0.0225	0.995
Indomethacin	0.14	0.21	29.55	0.6	0.2	0.085–0.360	0.999
Mefenamic acid	0.42	0.50	33.09	0.5	0.2	0.113–0.462	0.999

TABLE II

EXTRACTION EFFICIENCY

Drug	*Recovery (% of total)*			
	First extract	*Second extract*	*Third extract*	*Fourth extract*
Hydrochlorothiazide	72.9	23.2	3.9	0
Diazepam	65.0	30.0	4.5	0.6
Indomethacin	71.2	25.5	3.2	0.1
Mefenamic acid	66.8	29.6	3.2	0.4

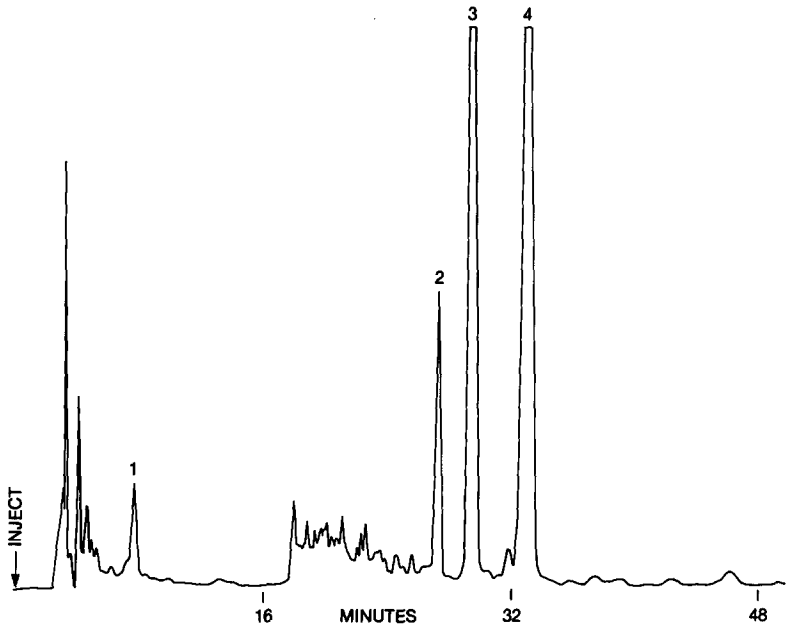

Fig. 1. HPLC profile of a Black Pearl pill containing hydrochlorothiazide (1), diazepam (2), indomethacin (3) and mefenamic acid (4).

Recovery of all four drugs was greater than 95%. The reproducibility of the analysis of tablet material was verified by determining the content of the four drugs in a finely ground homogeneous composite. The results (coefficients of variation) were 2, 6, 2 and 1% ($n = 6$) for hydrochlorothiazide, diazepam, indomethacin and mefenamic acid, respectively.

A chromatogram of the extract of a sample which contained the four added medicinal ingredients is shown in Fig. 1. A number of peaks due to unidentified substances will be noted. These may be other unidentified drugs or substances derived from the rather complex pill matrix.

One bottle containing twelve tablets was originally submitted for analysis. In all of the six tablets analyzed, the four drugs were found to be present. Fifty tablets taken from a different shipment were also submitted for analysis. From nine tablets analyzed, only two were found to contain the four drugs.

Subsequently, eight additional identical bottles of pills were received for analysis. One bottle contained 100 tablets and the remainder contained from 1 to 10 pills. From each bottle some pills (1 to 5) were analyzed quantitatively. The results of quantitative analysis of these eight batches of pills are shown in Table III, from which it can be clearly seen that there is a lack of homogeneity, suggesting a lack of quality

TABLE III

RESULTS OF ANALYSIS

Sample No.	*Hydrochlorothiazide (mg/tablet)*	*Diazepam (mg/tablet)*	*Indomethacin (mg/tablet)*	*Mefenamic acid (mg/tablet)*
1	2.50	0.47	5.88	7.79
	2.15	0.38	5.20	6.89
2	2.34	0.43	5.50	6.86
	2.15	0.36	5.39	7.21
3	1.94	0.41	5.41	7.14
	2.40	0.52	6.21	8.18
4	2.33	0.38	5.50	7.48
	2.26	0.42	5.75	7.91
5	2.05	0.44	5.85	7.56
	2.16	0.35	5.38	7.25
	1.82	0.40	5.28	6.98
6	2.00	0.47	6.18	8.21
7	–[a]	–	–	–
	–	–	–	–
	–	–	–	–
	–	–	–	–
8	1.64	0.35	4.09	5.19
	1.92	0.35	4.85	6.14
	1.36	0.30	3.69	4.54
	2.33	0.44	5.39	6.99
	2.34	0.35	4.74	5.88

[a] Not detected.

control in the manufacturing process. Sample 7 is different from the others in that no detectable amounts of added drugs were found.

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