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Report

Identification of Prescription Drugs in Adulterated Chinese Herbal Medications

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Analytical difficulties involved in the detection of a wide range of suspected polar and nonpolar drugs in botanical matrices have been solved by adopting a multipronged approach. Preliminary screening of Chinese herbal medications can be accomplished by a combination of thin-layer chromatography and gas chromatography/mass spectrometry and/or the employment of liquid chromatography/mass spectrometry as a probe introduction technique.

KEY WORDS: indomethacin; hydrochlorothiazide; mass spectrometry; *chuifong*.

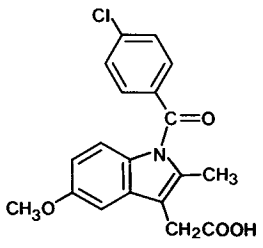
INTRODUCTION

Increased consumer usage patterns have recently developed for a myriad of herbal preparations. To avoid regulatory controls these products are often declared to be food supplements to escape the need for documented scientific proof of efficacy or safety. This situation is in direct contrast with public-health regulations governing the drug industry. New drugs have to undergo rigorous testing to support the manufacturer's claims to be safe and effective for intended therapeutic purposes. In the case of herbs, no such requirements exist, and the continuing chemical surveillance of such products is the only mechanism to ensure a measure of consumer protection.

While it is generally recognized that the drinking of herbal teas can cause toxic exposures via certain constituents such as the pyrrolizidine alkaloids (1), it is often difficult to establish firmly the exact causal relationship. These alkaloids are present as naturally occurring products in many of the herbs employed in blending herbal tea preparations. More recently, however, a new health hazard profile appeared when undeclared prescription drugs were found (2) in certain Chinese herbal medications (3). This appearance of a whole host of analgesic, antiinflammatory, and diuretic agents (to ensure medical claims) can be derived only by deliberate addition to the herbal products. In 1974 four cases of agranulocytosis resulting in the death of one person and extensive hospitalization of three others were linked to use of the preparations known as *chuifong toukuwan* (4). The pills involved in these cases were analyzed by the Food and Drug Administration (FDA) and found to contain phenylbutazone and aminopyrine. Investigations have shown that the pills originated from several foreign sources and entered the United States for distribution to health and oriental food stores, novelty shops, and individual consumers. Occasionally these products are sold door-to-door.

The analysis of such Chinese herbal medications represents multiple analytical problems. First, the presence of a complex mixture of botanical herbs as the matrix amidst the undeclared prescription drugs could offer a challenge in extraction, cleanup, and detection. Second, the prescription drugs used often differ greatly in chemical class and polarity and, therefore, can present difficulty if any one single separation technique is used for analysis. Third, the herbal medications do not usually conform to a well-organized quality-control situation and large variances in dosage may occur in the principal components as well as frequent drug substitutions.

We now report the most recent case history of the analysis of *chuifong toukuwan*, determined to contain significant levels of indomethacin (I) [1-(p-chlorobenzoyl-5-methoxy-2-methylindole-3-acetic acid], hydrochlorothiazide (II) [6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide], and chlordiazepoxide (III) [7-chloro-*N*-methyl-5-phenyl-3H-1,4-benzodiazepin-2-amine 4-oxide]. The analytical protocol selected demonstrates preliminary screening analysis by thin-layer chromatography (TLC), solvent extraction, liquid chromatographic separation, and finally, a cleanup step for structural confirmation by mass spectrometry.



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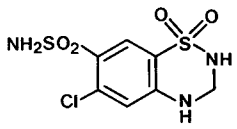

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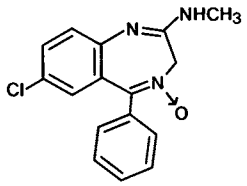

(III)

Scheme I

MATERIALS AND METHODS

Apparatus

Thin-Layer Chromatography. Precoated 20 × 20-cm TLC plates silica gel 60 F254 (EM Reagents) were employed using three different solvent systems: (A) methanol/chloroform/water (20:80:1) for indomethacin, hydrochlorothiazide, prednisolone, phenylbutazone, aminopyrine, acetominophen, and phenacetin; (B) methanol/chloroform (10:90) for phenylbutazone, antipyrine, dexamethasone, and hydrocortisone; and (C) chloroform (100) for phenylbutazone. After plate development with the appropriate mobile solvent, plates were viewed under short-wave UV light.

Liquid Chromatography. Equipment consisted of an Altex 110 pump, Rheodyne injector with 20-μl loop, Spheresorb-ODS 5-μm (25-cm × 4.6-mm) column, and SP8200 UV detector set at 254 nm, with a flow rate of 1.0 ml/min, areas integrated on an HP5880 integrator. For the mobile solvent system 0.7 g monobasic sodium phosphate and 0.7 g dibasic sodium phosphate were dissolved in 500 ml H_2O, adjusting the pH to 6.0 with 10% acetic acid; 300 ml of this solution was then diluted with 200 ml of acetonitrile.

Mass Spectrometry. Mass spectra were recorded on a Finnigan Model 3300 quadrupole mass spectrometer equipped with a chemical ionization source, an Incos data system, and a Finnigan liquid chromatographic moving belt interface.

Sample Preparation

Cleanup Procedure for Mass Spectrometry via MBI. In this procedure, 2 g Celite 545 was mixed with 1 ml pH 7.2 buffer solution and packed into a 25-cm × 200-mm chromatographic column with a small pad of glass wool above the column packing. The ground sample (0.7 g) was mixed with 2 ml pH 7.2 buffer solution and 3 g Celite 545 and then transferred to the column prepared above. Elution of the sample was accomplished with 150 ml chloroform previously saturated with water. The eluate was then evaporated just to dryness on a steam bath. The residue was dissolved in 25 ml 30% acetonitrile in H_2O, and the solution filtered through a 0.045-μm cellulose filter (solution 1). The column was then eluated with 150 ml ether previously saturated with water. This second eluate portion was also evaporated just to dryness on a steam bath. The residue was then dissolved in 30% acetonitrile in H_2O and filtered through 0.45-μm cellulose filter (solution 2). Extraction of solutions 1 and 2 was accomplished by adding 10 ml H_2O followed by three 25-ml portions of chloroform–acetonitrile (1/1). Extracts were then evaporated to dryness. Before analysis, the extracts were dissolved in 3 ml acetone.

RESULTS AND DISCUSSION

Thin-Layer Chromatography

As a primary screening technique, prepared extracts of the Chinese herbal medications were first examined by TLC to ascertain which undeclared prescription drug moieties might be present (Table I). The need for three different mobile phases of increasing polarity was experimentally derived in order to detect the wide range of drugs suspected to be present from previous case histories. As seen from Table I, the differentiation between indomethacin and phenylbutazone could be effectively achieved only by running in mobile-phase system A. This preliminary experimental approach provided valuable indications as to which undeclared prescription drugs should be further assayed and confirmed. In the case of extracts of *chuifong toukuwan* these TLC results indicated the presence of both indomethacin (I) and hydrochlorothiazide (II).

Gas Chromatography (GC)/Mass Spectrometry (MS)

While experience has indicated that the preliminary screening process provided by TLC is invaluable in detecting polar, thermally labile drugs, an additional routine screening of the extract was performed by conventional gas chromatography/mass spectrometry. The results of this adjunct approach usually indicated the presence of other constituent drugs unaffected by thermal degradation such as chlordiazepoxide (III) in the present study (Fig. 1). The protonated molecular ion at *m/z* 300 was a prominent ion, as well as the loss of 16 amu to give the ion at *m/z* 284. Ions at *m/z* 312 and *m/z* 328 then represented adduct ions formed with $C_2H_5^+$, to the protonated fragment ion at *m/z* 284 and the protonated molecular ion at *m/z* 300, respectively. The presence of protonated neutrals as well as their corre-

Table I. Layer Chromatographic Retention Data for Primary Drug Screening

Drug	Mobile-phase system[a] A	B	C
Acetaminophen	0.58		
Aminopyrine	0.88		
Antipyrine		0.69	
Dexamethazone		0.40	
Hydrochlorothiazide	0.43		
Hydrocortisone		0.50	
Indomethacin	0.92		
Phenacetin	0.88		
Phenylbutazone	0.97	0.97	0.12
Prednisolone	0.76		

[a] See Materials and Methods for details of mobile-phase systems.

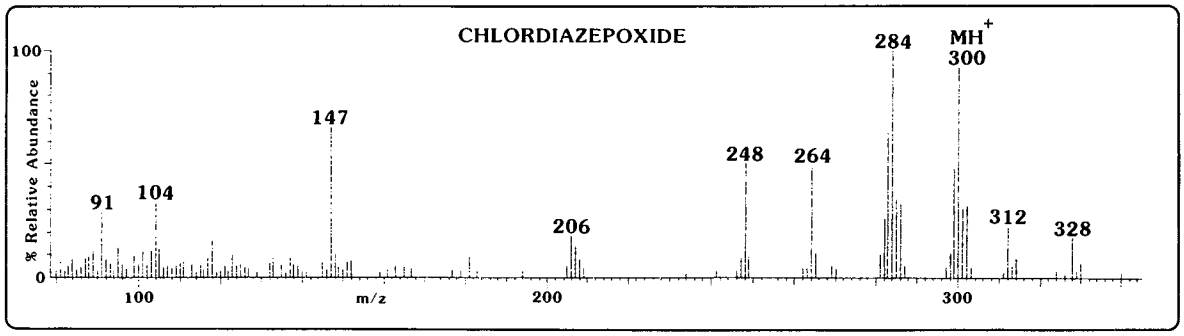

Fig. 1. Methane chemical ionization mass spectrum of chlordiazepoxide obtained under GC/MS conditions.

sponding adduct ions under chemical ionizations has recently been explained mechanistically as occurring via a proton bound bimolecular complex (5). Loss of HCl from the protonated molecular ion was indicated by the appearance of the ion at *m/z* 264.

Liquid Chromatography (LC)

Because of thermal lability, indomethacin and hydrochlorothiazide could not be characterized by conventional GC and were therefore analyzed by LC. Separation of these two drugs was accomplished using a Spheresorb-ODS column (Fig. 2). A number of other constituents of the extracts eluted shortly after injection but did not serious hamper quantitation by use of external standards. The assay values obtained from a composite sample of one lot of 20 pills were 2.6 mg/400 mg pill for indomethacin and 4.2 mg/400 mg pill for hydrochlorothiazide.

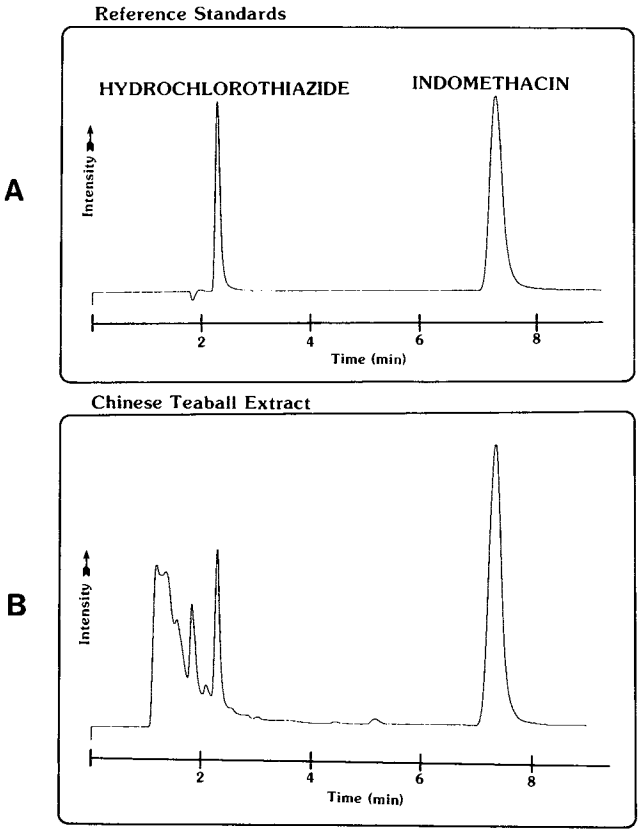

Fig. 2. Liquid chromatograms obtained for (A) reference standards and (B) extract of Chinese herbal medication.

Liquid Chromatography/Mass Spectrometry

To confirm the presence of both indomethacin and hydrochlorothiazide, a different sample extraction and cleanup protocol was used that separated the drugs of interest into two different eluates for mass spectrometry. The two resultant extracts were then examined using methane chemical ionization and the liquid chromatographic moving belt interface (MBI). Thermal degradation of the two drugs was minimized by spotting the eluates onto the belt for introduction to the source of the mass spectrometer (6). Further, the use of chemical ionization to produce protonated molecular ions for molecular weight determination of the compound under investigation is considered important for unambiguous confirmation (7). As illustrated in Fig. 3, the spectra obtained were found to be totally consistent with standard reference materials and represented the first published total mass spectral data recorded under CI conditions. No additional ions were present that might have interfered with this identification process. For indomethacin, the protonated molecular ion at *m/z* 358 was pronounced in the spectrum, with the base peak at *m/z* 139 corresponding to the cleavage of the C–N bond to give the 4-chlorobenzoyl cation. In both these ions, the evidence for the presence of one chlorine atom was clear. In the case of hydrochlorothiazide, the base peak was the protonated molecular ion at *m/z* 298, with a fragment ion at *m/z* 281 corresponding to loss of NH_3. This application of the moving belt interface as a probe sample introduction mechanism offers further advantages. With the two drug entities in question, the solvent system employed would have been difficult to adapt for direct introduction, and the choice of chemical ionization reagent gas limited. Selection of methane as reagent gas can often optimize conditions for the observance of protonated molecular ions, which are paramount to structure identification and confirmation.

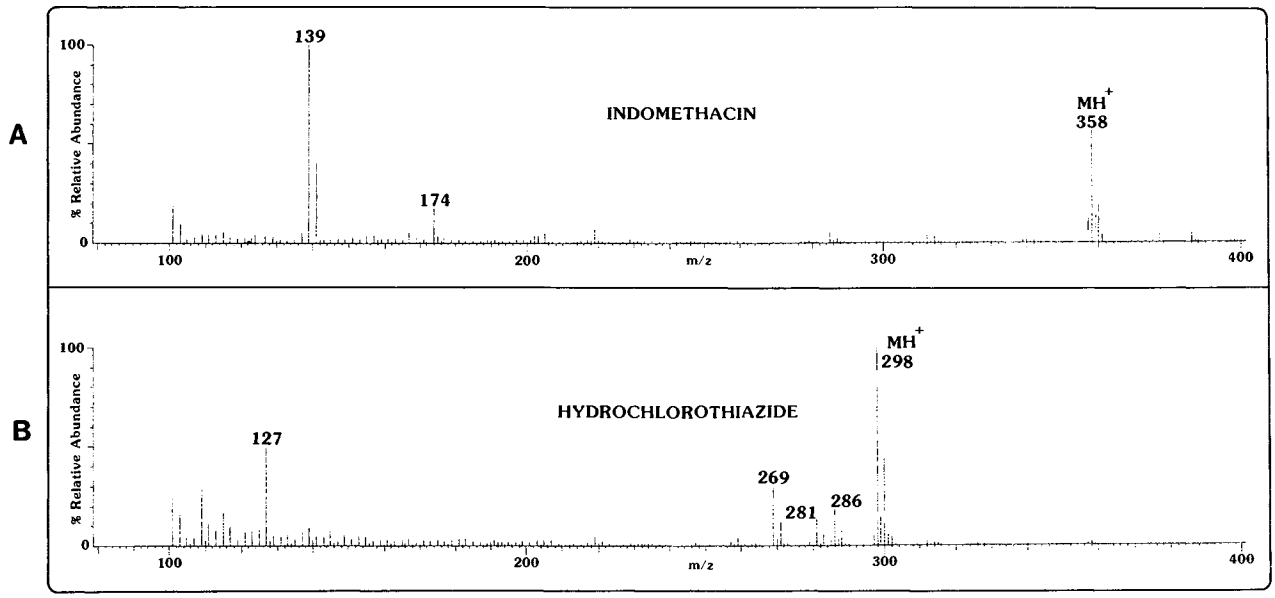

Fig. 3. Methane chemical ionization mass spectra obtained from actual sample eluates being spotted onto the liquid chromatographic moving belt interface for (A) indomethacin (**I**) and (B) hydrochlorothiazide (**II**).

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