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Screening Methods for Drugs and Heavy Metals in Chinese Patent Medicines

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The increase in health care costs coupled with the skyrocketing prices for prescription drugs in the United States has forced some individuals to search for alternative remedies for treating their ailments. In San Francisco communities where over-the-counter (OTC) Chinese Patent Medicines (CPM) from Asia are readily available to the public and at affordable prices, more people, other than ethnic minorities are turning to these cheaper alternatives. CPM are extracts of herbal (botanical, animal and mineral origins) formulae in the form of pills, tablets, and/or liquids. According to one study back in 1993, 3% of the general United States population use herbal products (Eisenberg et. al. 1993). However, more recent survey indicated that 12% to 37% of US consumers have used herbal medicines (Eisenberg et. al. 1998). A significant fraction of senior citizens, who are more disease prone and usually have limited financial resources, choose to resolve their medical problems by using these economical options. Unlike U. S. Food and Drug Administration (FDA) regulated drug products manufactured and distributed in this country, CPM are neither manufactured according to Good Manufacturing Practices (GMP) nor do they have quality control (QC) guidelines for their production. The listing of ingredients in CPM is frequently incomplete, and it is not unusual to find some undeclared drugs in them. In addition, heavy metals are found in CPM in varying amounts either because they are in the raw ingredients used or are introduced during formulation. The adverse effects of toxic levels of heavy metals are well established. It is the mission of the Food and Drug Branch (FDB) in the Department of Health Services to protect all Californians from exposure to illegal or harmful drugs. Therefore, in 1995 the Food and Drug Branch (FDB), and the Food and Drug Laboratory Branch (FDLB) have initiated a cooperative project to screen a large number of readily available CPM for drugs and heavy metals that may or may not be declared on the label.

In this drug screen study, 134 compounds were selected because they are the drugs and chemicals commonly found in the CPM sold in the United States. Lead, arsenic and mercury were chosen because they are used in traditional formulations and, at high concentrations, are known to have detrimental effect on humans (Beige1 et. al., 1998, Mitchell-Heggs et. al., 1990, Kang-Yum and Oransky 1992). Existing methods for drug screening are usually cumbersome and

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time consuming (Ng 1991;Yuen and Lau-Cam 1985; Taiwan Executive Branch 1994; Cairns et al. 1988; Johnson 1998). Therefore, we have devised an efficient and effective way to analyze the 134 specifically targeted drugs and chemicals (Table 1) by using Gas Chromatography/Mass Spectrometry (GC/MS). With this method, we are able to identify and confirm the presence of a wide variety of drugs and other toxic chemicals. We also used Atomic Absorption Spectroscopy (AA) to detect the presence of the three heavy metals. With the increase in use of herbal medicines, the need for product safety testing also increased, and the main purpose of this manuscript is to share this simple and efficient drug and heavy metal screen analyses with our colleagues.

The objectives of this project are to detect any undeclared drugs, lead, arsenic, or mercury that may be present in the CPM, and to establish a database for the CPM. This is an on-going study within the California Department of Health Services (CDHS) for the last five years. Since 1995, we screened over 500 CPM samples, and representative results are presented here. The data-base generated by this study has been used as an informative reference to educate the public, the herbal industry and the medical professionals.

MATERIALS AND METHODS

Omni-solv chloroform and methanol, reagent-grade ammonium hydroxide were used in drug screen, while magnesium nitrate, monobasic ammonium phosphate, redistilled concentrated nitric acid from GFS Chemicals, Inc. (P. O. Box 245, Powell, OH 43065) and J.T. Baker (222 Red School Lane, Phillipsburg, NJ 08865) CP grade 30% hydrogen peroxide were used for the metal screen. All drug standards were purchased from either US Pharmacopoeia or from Sigma - Aldrich Chemical Co. Certain reagents used in this study are considered to be carcinogens, therefore, appropriate safety precautions should be taken in the handling of those reagents.

Porcelain mortar and pestle, Whatman # 1 and # 54 filter papers, Kimax Squibb pear-shaped 60-mL separatory funnels, Kimax 58° chemical funnels, short stem' Nalgene 50-mL Teflon PFA flasks, Nalgene 100-mL Teflon PFA beakers, Thermolyne Model Cimarec 3 hot plate were used for preparation of samples. For the GC/MS analysis, we used the Hewlett Packard (HP/Agilent Technologies, 2850 Centerville Road, Willington, DE 19808) model 5971A Mass Spectrometer interfaced with HP 5890 Gas Chromatograph equipped with a data system which consisted of HP (Rev. C.03.00) Chemstation software. A 30 meter DB-5MS capillary column 0.25 mm id, 0.25 µm film thickness was used. For AA analysis, a Zeeman Atomic Absorption Spectrophotometer , the Perkin Elmer (Perkin Elmer Instruments, 761 Main Avenue, Norwalk, CT 06859) Model 5100PC Graphite furnace with Perkin Elmer AS 60 Autosampler, and an Atomic Absorption Spectrophotometer, namely the Perkin Elmer Model 3100 with Mercury Hydride System Model MHS- 10 were used.

Ten pills or tablets of the selected CPM were weighed to determine the weight per unit, and then were ground using a mortar and pestle. A minimum of 0.5 g (an approximate equivalent of 1 unit) of sample was used for the analysis of acidic and neutral drugs. The 0.5 g ground pill or tablet composite was extracted with 25 mL of methanol (Widdop 1986). The extract was filtered through Whatman # 1 filter paper. One µL of the filtered extract was injected into a gas chromatograph (GC) equipped with a mass spectrometer (MS). To analyze for basic drugs, a minimum of 0.5 g of the ground pills/tablets was weighed. Five ml of 5%NH_4OH (v/v) was added and this was extracted with 25 mL of chloroform using a separatory funnel (Widdop 1986). The chloroform layer was filtered through Whatman # 1 filter paper, and one µL of the filtrate was injected into the GC equipped with a MS.

Gas chromatography/mass spectrometry (GC/MS) analysis was performed using the following conditions. The temperature of the GC oven was held at 70 °C for 1 minute, then increased from 70 °C to 300 °C at a rate of 20 °C per minute, and then held at 300 °C for 15 minutes. The linear velocity of the helium gas was 35 cm/set. The ion source was operated at 190 °C; the transfer line and injection port were maintained at 300 and 250 °C, respectively. The mass spectrometer conditions were as follows: All analyses were carried out in the full scan electron impact mode with the mass range from 29 to 450 amu, and the electron multiplier voltage set at 200 V above autotune value. For detection purposes, 1 mg of drug standard was added to 0.5 g of sample prior to the extraction process. Methanol and chloroform were used as extracting solvents. Due to the low sensitivity of diphenoxylate, Nalidixic acid, phenytoin, probenecid, stanozolol, theophylline, naproxen and dipyrone, 5 mg of the drug standard was added to 0.5 g of sample. For liquid samples, 1.0 ml of the sample was used.

For metal analyses, one gram of ground sample was weighed and placed into a 100-mL Teflon beaker. Ten mL of redistilled concentrated nitric acid was added to the beaker. The sample was heated to boiling on a hot plate for 30 minutes. The sample was cooled and 1.0 mL of 30% hydrogen peroxide was added. It was further heated to boiling for another 20 minutes. The acid and hydrogen peroxide digestion steps were repeated twice. The digestate was diluted with 20 mL of 0.5% nitric acid and filtered through Whatman # 54 paper into a 50-mL Teflon volumetric flask. The beaker was rinsed with 0.5% nitric acid three times. The rinsed acid was added to the same 50-mL Teflon volumetric flask, and then filled to volume with 0.5% nitric acid. AA analysis was performed with the 20 µL of filtered digestate for lead, 25 µL for arsenic and 100 µL for mercury.
Atomic absorption (AA) analysis of lead was performed using a Perkin Elmer Model 5100 PC graphite furnace with an AS 60 autosampler. The parameters for the lead analysis were as follows: 283.3 nm wavelength, 0.7 nm low slit, hollow cathode lamp, charred at 850° C, atomized at 1800° C. The modifier used was 0.2 mg of ammonium phosphate and 0.01 mg of magnesium nitrate per injection (Perkin Elmer 1985). AA analyses of arsenic and mercury were done by using the

Perkin Elmer Model 3100 with Mercury Hydride System, Model MHS-10. The parameters for the arsenic analysis are as follows: 193.7 nm wavelength , 0.7 nm high slit, electrodeless discharge lamp, 3% sodium borohydride and 1% sodium hydroxide as reductant, and diluent was 1.5% hydrochloric acid (Perkin Elmer 1986). The parameters for mercury analysis were as follows: 253.6 nm wavelength , 0.7 nm high slit, hollow cathode lamp, and with the rest identical to those listed for arsenic (Perkin Elmer 1986).

RESULTS AND DISCUSSION

Table 1 shows the 134 targeted drugs and chemicals for which the standards were available, and could be detected by this method. Table 2 highlights some of our positive findings of the analyses of the 500 plus CPM samples. Approximately 10% of the tested CPM were found to contain undeclared drugs and/or toxic levels of lead, arsenic or mercury. Table 2 is a list of partial selections from the Compendium of Asian Patent Medicines (Ko and Au 1998). Of the 19 samples listed there, 15 tested positive for drugs and chemicals, 15 tested positive for lead, 9 tested positive for arsenic, and 5 tested positive for mercury. The amount of lead found ranged from 1 ppm to 184 ppm (0.018%), while arsenic from 68 ppm to 114,000 ppm (11.4%), and mercury was found to range from 329 ppm to 5070 ppm (0.507 %). Borneo1 and camphor are frequently found in the CPM samples tested (data not shown). Other chemicals of interest (not listed in Table 1), sometimes harmful in nature, are also detected in this screening method. Of significance, 5-fluorouracil, an anti-neoplastic agent, was found in the product named Capsulae Tegafuri Compositae.

Of the 500 OTC medicines tested, we found approximately 10% contained undeclared drugs and/or toxic levels of lead, mercury or arsenic. The United States Pharmacopoeia limits heavy metals in most oral pharmaceuticals to 30 ppm, with lower limits for lead, arsenic and mercury (Ko 1998). Even though GC/MS provides us with confirmed findings of undeclared drugs, there are limitations to the use of GC/MS for the screening of OTC CPM. Non-volatile chemicals, large organic chemicals, and inorganic substances are not detected by GC/MS under the above mentioned conditions. Penicillin, for example, cannot be detected. In this study, we focused our efforts on the volatile and medium size drugs (i.e. molecular weigh less than 450), and have established the list of 134 targeted compounds according to these criteria. Therefore, negative analytical results (data not shown) do not necessarily indicate that the products have no undeclared drugs. It can only be stated that the sample is free of the 134 targeted drugs and chemicals at concentrations equal to or greater than the levels detectable by this method. Many pharmaceutical drugs are derived from natural products, and it is possible that the chemicals detected in these products are from natural origin and are not intentionally added (Huang 1993). Lead, arsenic and mercury are detected by AA. The sensitivity of the assay allowed the detection of low levels of the three heavy metals. Lead can be a potential contamination of the

Table 1. Screened Drugs and Chemicals

Acetaminophen	Ethopropazine	Phenacetin
Alprazolam	Ethosuximide	Phenazopyridine.HCl
Aminopyrine	2-ethoxybenzamide	Phenformin
Amitriptyline	Fenfluramine	Pheniramine
Amphetamine	Flurazepam	Phenmetrazine
Atropine	Furazolidone	Phenobarbital
Baclophen	Glutethimide	Phentermine
Benztropine	Griseofulvin	Phenyl Salicylate
Bergapten	Guaifenesin	Phenylbutazone
Betamethasone	Haloperidol	Phenylpropanolamine
Borneol	Heptaminol	Phenytoin
Bromazepam	Hexachlorophene	Prednisolone
Brompheniramine	Homatropine	Probenecid
Caffeine	Hydrocodone	Procaine
Camphor	Imipramine	Progesterone
Carbamazepine	Lapachol	Promethazine
Carbinoxamine	Loratadine	Propantheline Bromide
Carisoprodol	Lovastatin	Propoxyphene
Chlordiazepoxide	Mazindol	Propranolol
Chloroquine	Meclizine	Prozac (Fluoxetine)
Chlophedianol	Mefenamic Acid	Pseudoephedrine
Chlorpheniramine	Menthol	Pulegone
Chlorpromazine	Mephenesin	Pyrilamine
Chlorpropamide	Mephentermine	Quinidine
Chlorzoxazone	Mestranol	Quinine
Clemastine	Methadone	Santonin
Clonaxepam	Methamphetamine	Secobarbital
Clonidine	Methimazole	Stanozolol
Cocaine	Methocarbamole	Strychnine
Codeine	Methyltestosterone	Sulfadiazine
Coumarin	Metoprolol	Testosterone
Cyclandelate*	Metronidazole	Testosterone Propionate
Cyclizine	Morphine	Tetrahydropalmatine
Cyclobenzaprine*	Nadolol	Theophylline
Dexamethasone	Nalidixic Acid	Thiabendazole
Dextromethorphan	Nalorphin	Tolbutamide
Diazepam	Naproxen	Triazolam
Dihydrocodeine	Nicotine	Trihexyphenidyl*
Diphenhydramine	Norethindrone	Trimethobenzamide
Diphenidol	Nortriptyline	Trimethoprim
Diphenoxylate	Orphenadrine*	Tripelennamine
Dipyrone	Oxazepam	Triprolidine
Doxylamine	Oxycodone	
Ephedrine	Oxymetholone	
Estradiol	Pentazocine	*FDL in-house library
Estrone	Pentobarbital	instead of Wiley Library

Table 2. Chinese Patent Medicines with Detectable Levels of Drugs and Chemicals

Product Name	Lead (ppm)	Arsenic (ppm)	Mercury (ppm)	Drugs/Chemicals Detected
An Kung Niu Huang Wan (Bezoar Chest Functioning Pills)	184±14	46400±900	1450±70	borneol, camphor
Bezoar Sedative Pills	14.3±1.0	68.5±1.4	5070±250	nd
Capsulae Tegafuri Compositae	nd	nd	nd	5-florouracil
Cogent 20	nd	nd	nd	phenacetin
Du Huo Jisheng Wan	4.4±0.3	nd	nd	acetaminophen
Fargelin (Yang Cheng Brand)	1.3±0.1	20200±400	nd	nd
"Gan Mao Qing" Capsule	2.8±0.2	nd	nd	acetaminophen, chlorpheniramine
Hiya Kiogan	65.4±4.9	93200±1900	nd	not tested
Huang Lien Shang Ching Pien	1.1±0.1	nd	nd	pulegone, phenacetin
Liteling Tablets (SPIC Brand)	nd	nd	nd	furazolidone
Lu Shen Wan	40±3.0	82000±2000	3850±190	resibufogenin*
Nan Lien Chuifong Toukuwan	2.2±0.2	nd	nd	diazepam, mefenamic acid, 4-methyl-5-thiazolethanol*
Niu Huang Xiao Yan Wan (Bezoar Antiphlogistic Pills)	21.2±0.1	114000±2000	329±16.0	nd
Pa Pao Ching Feng San	3.3±0.2	29200±600	nd	borneo, camphor
Pe Min Kan Wan (Chu Kiang Brand)	nd	nd	nd	chlorpheniramine
Peking Niu Hiang Chien Tu Pien	1.1±0.1	36900±700	nd	borneol
Sha Hee Pills	9.8±0.7	44800±900	2610±130	ephedrine, eugenol/isoeugenol*
Specific Lumbarglin	1.5±0.1	nd	nd	acetaminophen
Tong Shap Yee Asthma's Pills	1.3±0.1	nd	nd	aminophylline*, ephedrine, caffeine

nd = none detected

* Identified by library search on the mass spectra.

products, while arsenic and mercury are usually added as part of traditional formulations (Chinese Pharmacopoeia 1997). Arsenic and mercury are listed as herbal ingredients in the Peoples' Republic of China Pharmacopoeia and are labeled as realgar and cinnabar, respectively. Some of the CPM samples have identical brand names and labels but are manufactured in different regions of China. The data generated from these products can be vastly different in their content of ingredients, therefore, some of the results obtained are not identical. The data shown in Table 2 are from CPM samples we analyzed that contained either undeclared drugs or existing high concentrations of the three metals or both. Health departments in several of the Pacific Rim regions, e.g., Taiwan and Hong Kong, recently have conducted heavy metal contamination studies on some of the CPM, and have come to similar conclusion based on their findings (Chinese Pharmacopoeia 1997; Kang-Yum and Oransky 1992; Taiwan Executive Branch 1992; Taiwan Executive Branch 1993; Chan et al. 1993; Ko 1998).

The lack of uniform Good Manufacturing Practices in China and other Asian countries creates a safety problem for regulators who ensure the quality and safety of imported CPM. To ensure that the usage of CPM poses no public health hazard in California, the approach that the California Department of Health Services has taken is to educate the public and the industry about the potential dangers of some of the products. This will enable the consumers to make informed decisions on their choices. A bilingual (English - Chinese) compendium listing some of the results of our findings on CPM has been published and is available (Ko and Au 1998). The work presented in this paper is the result of the concerted effort of the Food and Drug Laboratory Branch and the Food and Drug Branch of the CDHS. The ultimate goal of this study is to disclose the possible causes of toxicity as well as to ensure the safety of readily available Chinese Patent Medicines

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