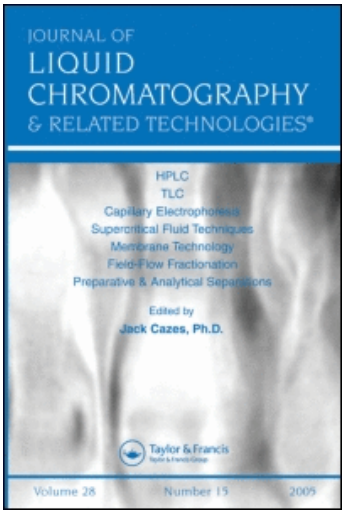

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Identification of Western Medicines as Adulterants in Chinese Herbal Medicines Using a Broad-Spectrum Drug Screening HPLC System

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To cite this Article Lai, S. Jason , Binder, Steven R. , Essien, Herbert and Wen, Kuo-Ching(1995) 'Identification of Western Medicines as Adulterants in Chinese Herbal Medicines Using a Broad-Spectrum Drug Screening HPLC System', Journal of Liquid Chromatography & Related Technologies, 18: 14, 2861 — 2875
To link to this Article: DOI: 10.1080/10826079508009330
URL: http://dx.doi.org/10.1080/10826079508009330

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(14), 2861-2875 (1995)

IDENTIFICATION OF WESTERN MEDICINES AS ADULTERANTS IN CHINESE HERBAL MEDICINES USING A BROAD-SPECTRUM DRUG SCREENING HPLC SYSTEM

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ABSTRACT

Analysis of herbal medicines is a difficult task because of the complexity and variety of the available formulations. Identification of adulterants in herbal medicines poses an even greater challenge to the laboratories which are required to conduct a routine surveillance program. There is no single broad spectrum screening method which will be able to screen all non-herbal medicine in a single run. However, it is logical to identify a few broad spectrum screening methods in order to cover the most frequently encountered drugs. REMEDi HS, a commercial system, has been evaluated for screening the neutral and basic synthetic medicines as adulterants in the herbal medicines. The system utilizes an on-line sample purification and cleaning procedures, and it requires minimal sample pre-treatment. The library used during this study contained 555 drugs and metabolites. Diazepam, chlordiazepoxide, sulpiride, etenzamide, caffeine and metoclopramide were identified in the five herbal medicines submitted from

consumer. The latter four medicines have not been reported before as adulterants in herbal medicines. All results were confirmed by GC/MS.

INTRODUCTION

Herbal medicines are rarely considered in analytical toxicology, even though about 70 percent of world population currently use some forms of herbal medicines [1]. Recently, the presence of adulterants in herbal medicines have been reported [2, 3]. For example, several steroid adulterants were reported to be present in herbal medicines in Pakistan[2]. A recent report indicated that an adulterated herbal pill (Chui Fong Tou Ku Wan) was sold in the US, and analysis of the tablets has shown the probable presence of a steroid (prednisone) and a benzodiazepine (diazepam) [3]. The US Food & Drug Administration has traced manufacturer of these illegal pills to Hong Kong [3].

In Taiwan, herbal medicine is subjected to the same routine surveillance as western pharmaceuticals under the jurisdiction of the National Laboratories Of Foods And Drugs (NLFD). The institute, which regulates foods, cosmetics, pharmaceuticals, medical devices and narcotics, also investigates consumer complaints such as the analysis of questionable products. Often, the adulterated herbal medicines are sold under the name of "wonder drug" without any indication of the content and the manufacturing location.

When considering the number of the commonly used western medicines (at least 1000 drugs) and the complexity of herbal medicine, the identification of the adulterants in the herbal medicines poses a great difficulty to laboratories. In addition, since drugs do cross borders, the analyst may be faced with unfamilar ingredients. The most difficult task for the laboratories that conduct routine surveillance of medicine content is to structure a manageable and systematic approach for an effective screening. Because of the great variation of chemical properties, it is not possible to devise a single screening method to cover all medicines. However, it is logical to seek a few complementary methods which can provide an adequate broad screen in a timely fashion. The fewer the methods required, the more effective the surveillance program will be.

Chromatographic methods are desirable because of their ability to identify a large number of drugs in a single analysis. Among various chromatographic techniques, Gas Chromatography (GC)[4-12], Thin Layer Chromatography (TLC) [13-15] and High Performance Liquid Chromatography (HPLC) [16-19] are commonly used for screening drugs. HPLC is a desirable method because it minimizes sample preparation.

A HPLC system equipped with a two-dimensional data analysis capability will provide a more definite identification than retention index alone. Two dimensional data analysis in HPLC combines chromatographic retention indices and spectrometric parameters (such as UV wavelength ratios or full UV spectral matching). Numerous examples of HPLC applications in western medicines can be found for the identifications of antidepressant [20], benzodiazepines [21, 22], neuroleptics [23], laxatives [24] and diuretics [25, 26]. This technique has also been used for analysis of vegetable products, such as the identification of active ingredients in the fruits of Gardenia Jasminoides Ellis and Gardenia Jamsminoides Var. Grandiflora Nakai [27], the analysis of active ingredients of medical plants such as Ginseng [28].

Sample treatment to remove the unwanted sample matrix is a required step in this type of analysis, mainly to eliminate interference. Off-line extraction procedures are commonly used in GC and HPLC drug screening. However, an on-line extraction and purification procedure will permit automation of the analysis process. Previously, we have reported that a HPLC system (REMEDi) for screening over 250 drugs in serum and urine matrix using on-line purification procedure [29-31].

Here we describe the application of an enhanced model of this HPLC system (REMEDi HS) for screening neutral and basic drugs in the adulterated herbal medicines with minimal sample preparation.

MATERIALS AND METHODS

Samples

Five herbal samples were analyzed. Two were in the powder form, and three were in the form of pellets. These five samples submitted as part of rountine consumer inquiries to NLFD.

Sample Preparation For REMEDi HS

A 10 ml of methanol/water solution (50/50, v/v) was mixed with 100 mg herbal medicine (the pellet sample was crushed and grinded before weighing) in a 20 ml sample vial. The solution was vortexed for 30 seconds and mixed on a rocker for 10 minutes.

Fifty μl of the top clear solution was transferred from the 20 ml vial to a microfuge tube. 950 μl 1% saline solution (1 g NaCl in 100 ml D.I. water) and 200 μl of Internal Standard solution were added to the microfuge tube and mixed well by vortexing for 10 - 15 seconds.

The tube was placed in a microfuge and spun for one minute at 9,500 xg. 1.0 ml of the supernatant was used for the analysis.

REMEDi HS & Reagents :

The configuration of the HPLC system (REMEDi) and mobile phase were described in a previous report [29]. In this study, an enhanced system was used (REMEDi HS™, BIO-RAD Laboratories, Hercules, CA, USA). The injection volume has been increased from 0.5 ml to 1.0 ml. Additionally, the light path of the flow cell has been changed from 5 mm to 10 mm. For most of the drugs evaluated, the identification limit was 100 to 300 ng/ml. The library contained 555 drugs and metabolites (software version 4.12). A 486 ALR computer was used.

Figure 1 depicts the analysis steps by REMEDi HS, including on-line sample preparation, analytical separation, scanning UV detection, evaluation of spectral data, retention data & calculation of concentrations (when required) and a comprehensive report. The HPLC system consists of 5 cartridges and 4 switching valves, and it uses 5 reagents. The internal standards used were Ethylnordiazepam (IS1) and Chlorpheniramine (IS2).

GC/MS :

A Hewlett-Packard 5989A GC/MS Engine (Palto Alto, CA, USA) was used for the study. A J&W Scientific (FISONS, Folsom, CA 95630) DB-5

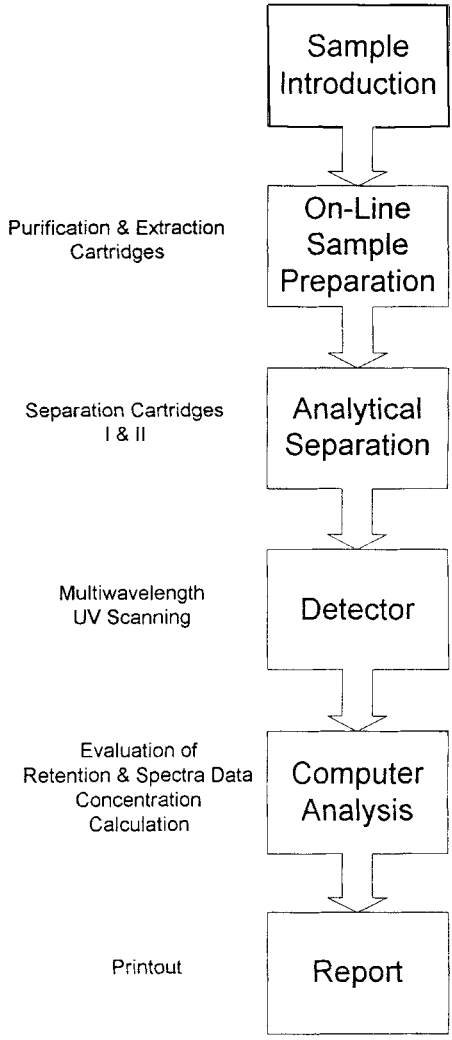

Figure 1. REMEDi HS Schematic.

capillary column (15 m, 0.25 mm I.D., 0.25 microns film) was used. Temperature program was set at 80 ^{o}C for 1 minute, then increased to 280 ^{o}C at a rate of 15 ^{o}C/min, and maintained at 280 ^{o}C for 11 minutes. The injection volume was 2 μl.

Sample Preparation For GC/MS Using Solid Phase Extraction

The 200 mg Clean Screen Extraction Column from United Chemical Technology (Horshma, PA 19044, USA) was used for solid phase extraction for GC/MS. The procedure for "Therapeutic And Abused Drugs In Urine For GC Or GC/MS Confirmation (DRU200DAZ120392)" from United Chemical Technology was used. A sample volume of 2 ml was used. The final volume of extracts was 50 μl in methanol. All organic solvents used in solid phase extraction are GC/MS grade.

RESULTS AND DISCUSSION

Formulation And Analysis Of Chinese Herbal Medicine

Chinese herbal medicines has been used in the Asian region for several thousands of years, and even today it is still the predominant medical practice in this region, including Taiwan, mainland China, Korea, Japan and southeastern Asian countries. The majority of the herbal formula are well documented and provided with a quantitative composition. The early written publications [32, 33] can be traced back as far as two thousand years ago in Chinese Han Dynasty (B.C. 202 - A.D. 220), 113 formulas were recorded in the book of Shang Han Lun and 110 formulas in the book of Chin Kuei Yao Lueh. Since then, the number of recorded formulas grew rapidly. About thirteen hundred years ago during the Chinese Tang Dynasty (A.D. 618 - A.D. 755), there were over 6,000 formulas in the book of Wai Tai Mi Yao; during the Chinese Sung Dynasty (A.D. 960 - A.D. 1279), 30,000 formulas were recorded in the book of Tai Ping Sheng Huei Fang; in Chinese Ming Dynasty (A.D. 1368 - 1644), there were over 60,000 formulas in the book of Pu Chi Fang; and in Chinese Ch'ing Dynasty (A. D. 1644 - 1911), there were over 8,000 formulas in the book of Pen Tsao Kang Mu. The total known herbal formulas exceed 100,000 when counting additional formulas from other medical compendia. Among this

huge number of formulae of Chinese herbal medicines, about 1,200 are commonly used, and 300 of them have been thoroughly analyzed [33]. About 200 of them are presently used in Japan, and 116 are available as over-the-counter medicines by TSUMURA Pharmaceutical(Tokyo, Japan).

In 1888, contemporary scientific investigations began to be conducted on Chinese traditional medicine, and paeonol was first isolated by Nagai from the root bark of Paeonia moutan Sim which is used as a Chinese crude drug under the name of Mu-tan-phi[34, 35]. In the oldest Chinese Materia Medica, Shin-rung Pen T'sao Ching, 365 crude herbal drugs were recorded [34], and recently 60 of them were studied extensively and the chemical structures of the major ingredients were identified [36]. Except for a few herbal formulas containing only a single herbal plant, most of the herbal medicines are multiple ingredient formula, which usually include eight to twelve different herbal plants. The analytical measurement of herbal medicines is a great challenge to most laboratories. Presently, the available published research literature only describes a small portion of the existing herbal medicines.

Identification

The sample solutions of the 5 adulterated herbal medicines had a dark brown color, and they were applied to the system directly with a minimal sample preparation. The REMEDi HS uses an on-line approach to remove the unwanted herbal sample matrix, while allowing analysis of a wide variety of medicines with greatly different chemical properties. The identification is made by a two-dimensional data analysis immediately after the separation. Up to 50 samples can be continuously analyzed, and the whole process is carried out automatically. Figure 2 is a typical chromatogram of an herbal sample by REMEDi HS. Table 1 lists the identified western medicines and their quantities in these five adulterated herbal medicines, including chloridiazepoxide, sulpiride, etenzamide, metoclopramide, diazepam and caffeine. GC/MS confirmed the presence of these six drugs. Their retention times in GC/MS were 6.84 minutes for etenzamide, 8.54 minutes for caffeine, 12.46 minutes for diazepam, 12.96 minutes for chlordiazepoxide, 13.73 minutes for metoclopramide and 16.32 minutes for sulpiride. Sulpiride was analyzed as the trimethylsilyl derivative.

DATE : 03/29/94 TIME: 21:16 hrs METHOD: TI.QNT VOLUME:1000
SAMPLE ID: BR1621 INJ # 50870 OPERATOR ID: TI
COMMENTS : VIAL # 4

PEAKS DETECTED

IDENTITY	NOTES	PEAK#	RT	L-MAX	PEAK-HT	W-L
		1	1.11	269	22953	235
		2	1.29	234	72773	205
		3	1.45	233	29287	205
		4	1.93	UNKNOWN	7592	205
CHLORDIAZEPOXIDE[-s-]	W1	5	2.14	271	49225	205
		6	2.45	UNKNOWN	6530	205
IS1[Nordiazepam,N-ethyl]		7	3.35	233	563829	205
Sulpiride		8	10.06	214	286975	205
IS2[Chlorpheniramine]		9	11.28	229	276098	205

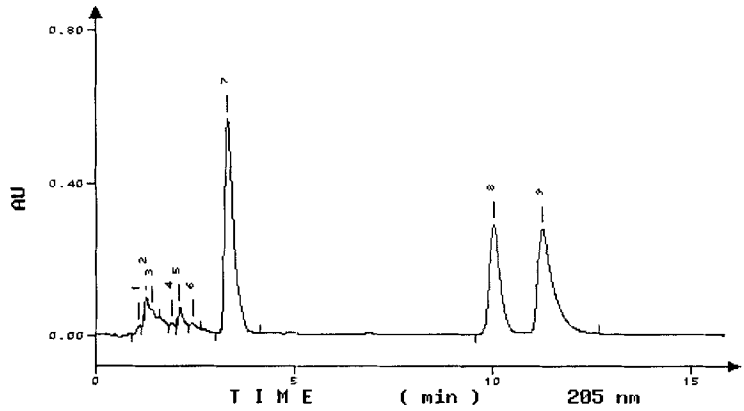

Figure 2. A Chromatogram Of An Adulterated Herbal Medicine Sample. Peak 5 : Chlordiazepoxide; Peak 7 : Internal Standard 1 (Ethyl-nordiazepam); Peak 8 : Sulpiride; Peak 9 : Internal Standard 2 (Chlorpheniramine).

Table-1 Adulterants Found In The Herbal Samples

No.	Form	REMEDi	GC/MS	Concentration	Class
1	Powder	Chlordiazepoxide	Chlordiazepoxide	1.01 mg/g	Tranquilizer
		Sulpiride	Sulpiride	12.16 mg/g	Tranquilizer
2	Pellet	Diazepam	Diazepam	0.33 mg/g	Tranquilizer
		Etenzamide	Etenzamide	10.75 mg/g	Analgesic
3	Powder	Metoclopramide	Metoclopramide	0.38 mg/g	Anti-emetic
4	Pellet	Diazepam	Diazepam	0.53 mg/g	Tranquilizer
		Etenzamide	Etenzamide	5.64 mg/g	Analgesic
		Caffeine	Caffeine	12.38 mg/g	Stimulant
5	Pellet	Diazepam	Diazepam	0.87 mg/g	Tranquilizer

Table-2 Reproducibility At the Detection Limit of REMEDi HS

Drug Name	R.T.	CV%	RRT2	CV%	Pk Ht	CV%	Detection Limit (ng)
Chlordiazepoxide	2.14	0.27	0.082	0.71	19083	2.38	100
Diazepam	2.92	0.20	0.156	0.37	28205	3.44	100
Etenzamide	1.82	0.37	0.037	3.07	292843	8.71	500
Metoclopramide	9.33	0.06	0.833	0.37	20807	7.26	200
Sulpiride	10.18	0.10	0.894	0.06	32128	0.46	300
Caffeine	1.43	<0.001	0.006	<0.001	74747	5.53	500

* Sample matrix is a solution of 1% Saline/Methanol (95/5; v/v). n=3.

Two Dimensional Data Analysis

The identifications of these compounds are made by comparing their retention indices and UV spectrum data. Table 2 lists the reproducibility of the retention data and the peak heights of these six drugs, and their identification limits are also tabulated. For REMEDi, three wavelength ratios are used as well as a similarity factor (SF) which measure the matching fitness of two UV spectra throughout the measured UV range (205 nm to 300 nm).

The figure 3 shows the UV spectrum of these six drugs. Table 3 lists the wavelength ratios for these six drugs.

Quantitation

Calibrators were prepared in 1% saline. Table 3 shows the linearity data of the six adulterants identified in these five herbal samples.

The Adulterants in Herbal Medicines

Among the six adulterants reported here, sulpiride, etenzamide, caffeine and metoclopramide were not reported previously.

Diazepam is one of the well-known adulterants in the herbal medicines [3]. It is a benzodiazepine type of tranquilizer, and it is frequently prescribed for the management of general anxiety disorders, panic disorders, and to provide

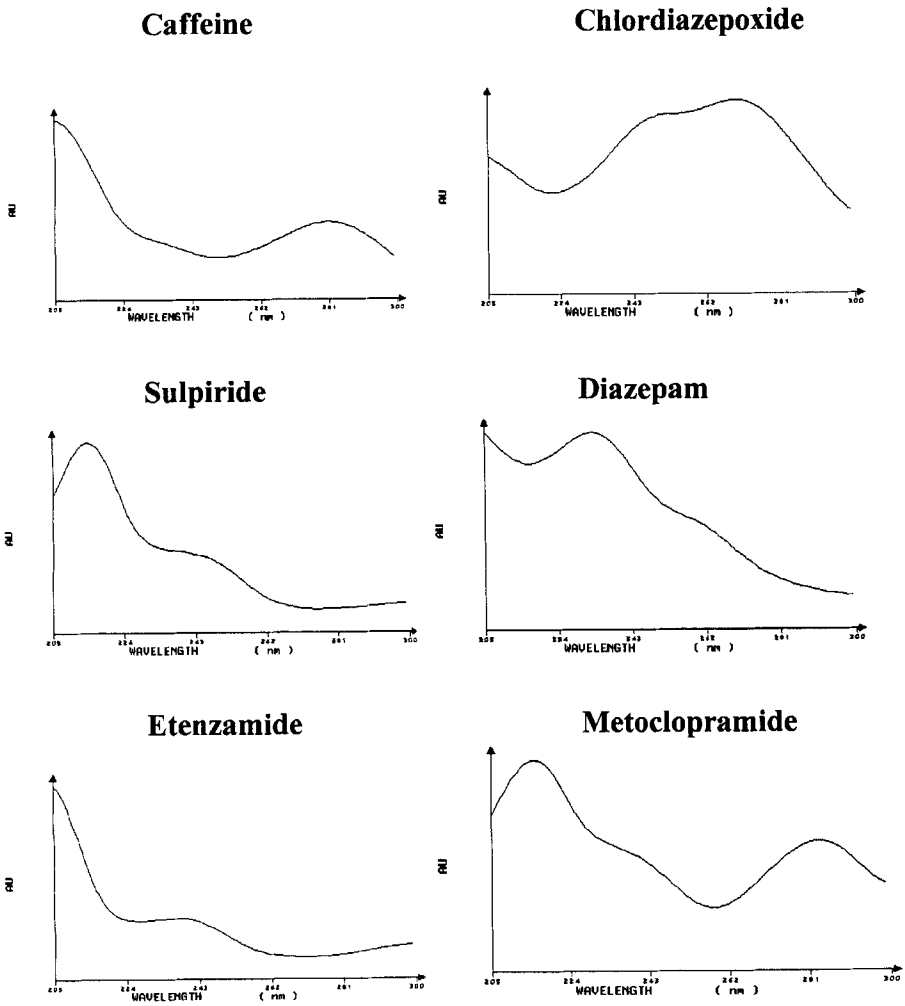

Figure 3. UV Spectra Of Caffeine, Chlordiazepoxide, Sulpiride, Diazepam, Etenzamide, And Metoclopramide.

Table-3

Drug Name	Conc (ug/ml)	Mean Peak Height (n=3)	CV%	Linearity Fit (r value)
Chlordiazepoxide	0.10	19083	2.38	0.999936
	2.00	330479	1.47	
	4.00	652407	0.40	
Diazepam	0.10	28205	3.44	0.999919
	0.50	142351	0.54	
	1.00	278811	0.08	
Etenzamide	0.50	292843	8.71	0.999712
	1.00	505019	5.60	
	1.50	735598	3.87	
Metoclopramide	0.20	20807	7.26	0.999862
	2.50	241525	0.36	
	5.00	481487	0.49	
Sulpiride	0.30	32128	0.46	0.999481
	2.50	255007	0.37	
	5.00	481487	0.36	
Caffeine	0.50	74747	5.53	0.999984
	1.00	155718	6.41	
	5.00	766694	1.18	

* Sample matrix is a solution of 1% Saline/Methanol (95/5; v/v). n=3.

preoperative sedation, light anesthesia, and amnesia, treatment of epileptics, alcohol withdrawal symptoms, skeletal muscle relaxant. Benzodiazepines are easily subject to abuse and development of tolerance and dependence [37].

Chlordiazepoxide is also a benzodiazepine, and it was reported previously in an adulterated chinese herbal medicine [38] . This particular chlordiazepoxide-containing herbal sample also has a high dosage of sulpiride (12.16 mg/g), which is usually administered as an antidepressant, an antipsychotic agent or a digestive aid. The combination of sulpiride and chlordiazepoxide is similar to the formulation of a commercially available neurosis medicine (trade name, Anisum), which contains sulpiride and diazepam.

Sulpiride is reported to have mood elevating properties, and has anti-emetic actions and an effect on gastric secretion. It is commonly used in this region for this purpose, and it has an detection rate of about 1% in a recent study of the patient samples from emergency room in this region [39].

Etenzamide was found in two herbal samples (#2 & #4). Etenzamide, an analgesic agent, is rarely used in the USA, but is often used as a pain killer in Japan and nearby countries. A formulation of western medicine was reported to contain etenzamide (Ethoxybenzamide), acetaminophen, bromvalurea and caffeine, manufactured by Mintong Pharmaceutical (Taiwan). In these two samples, Sample 2 contains diazepam, and Sample 3 contains diazepam and caffeine. Caffeine can increase mental alertness, and is often included in the formulations containing aspirin, acetaminophen, or codeine [40].

Metoclopramide stimulates motility of the upper gastro-intestinal tract and is used for the management of some forms of nausea and vomiting, in gastro-oesophageal reflux, and gastric stasis [41]. In one study conducted in Taiwan, it was detected in 1.2% in the patient samples collected in the emergency department [39].

Most of the adulterants identified in this report belong to the category of pain relievers. The adulterants are usually pure chemical components which can be rapidly absorbed by the human body, and the patient will have the sensation of instant relief. This type of adulterated drug is commonly sold under the name of "wonder drug", or it is added as an "undisclosed" ingredient to a regular herbal medicine by unlawful manufacturers. Because herbal medicine has been integrated with Asian medication history and daily life for many thousands of years, the adulterants in the form of herbal medicine are less likely to be noticed by the patient and they are difficult to detect in the herbal matrix.

CONCLUSION

Six adulterants are identified in herbal medicines, including diazepam, chlordiazepoxide, sulpiride, etenzamide, metoclopramide and caffeine. All results were confirmed by GC/MS. Except diazepam and chlordiazepoxide, the other four drugs were not previously documented. These six drugs belong to four classes of drugs : analgesic(etenzamide), anti-emetic (metoclopramide), xanthine stimulant (caffeine) and tranquilizers (diazepam, chlordiazepoxide & sulpiride). The findings which we report here demonstrate that the identification of each individual drug from the screening method can expedite the confirmation process. Specifically, four out of these six drugs (etenzamide, metoclopramide, sulpiride and caffeine) could not be screened by immunoassays, nor are they routinely analyzed by a single chromatographic method.

ACKNOWLEDGMENT

The authors thank Mark Yeh for coordinating sample transportation and Tadashi Itagaki for technical assistance.

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Received: February 23, 1995
Accepted: March 7, 1995