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Gas Chromatography–Chemical Ionization Mass Spectrometry for Drug Screen Analyses

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In California, the proliferation of ethnic patent medicines available in health food stores and ethnic markets has created concerns among state agencies responsible for consumer safety, especially where illnesses or even death has been associated with the consumption of herbal remedies (Ko et al. 1996). To ensure the safety of the general public, it is imperative for the surveillance agency to be able to quickly screen over-the-counter remedies for potentially hazardous content.

An estimated 75% of pharmaceutical compounds are basic or possess basic character. Therefore, High Performance Liquid Chromatography (HPLC) has been a preferred analytical method for many pharmaceuticals, as detailed in The United States Pharmacopeia (USP/NF 1995). However, these procedures are usually time consuming and tedious. In a recent publication, we showed that it was possible to simultaneously screen for 134 targeted drugs in Chinese Patent Medicine (CPM) sample preparations using GC-MS (Au et al. 2000). However, as shown in Fig 1A and 2A, a number of the EI mass spectra revealed either insignificant or no molecular ions, or insufficient fragmentation patterns for conclusive confirmation of positive findings. (e.g. diphenoxylate, diphenidol, sulfadiazine, procaine, pentobarbital, methadone).

Since a defensible analytical result is a crucial legal standard for any violative findings, we examined the possibility of using Gas Chromatography-Chemical Ionization Mass Spectrometry (GC-CIMS) as a screening method in the analysis of CPM for cases such as adulteration or mislabeling of products. CIMS has been shown to provide complementary information to EI, particularly in molecular weight determination and structure elucidation. Cirimele and co-workers (1997) have described a procedure for the screening for 8 benzodiazepines in human hair by GC-negative ion chemical ionization-mass spectrometry after derivatization. To our knowledge, no prior studies have reported the comprehensive use of GC-CIMS for drug screening. With methane as the reagent gas and using GC-CIMS, we have tested a total of 134 drug/chemical standards for drug screen analyses.

MATERIALS AND METHODS

Except as specified, all drug/chemical standards were prepared at 40 ng/ul in methanol. Standards were purchased from either U.S. Pharmacopeia, Rockville,

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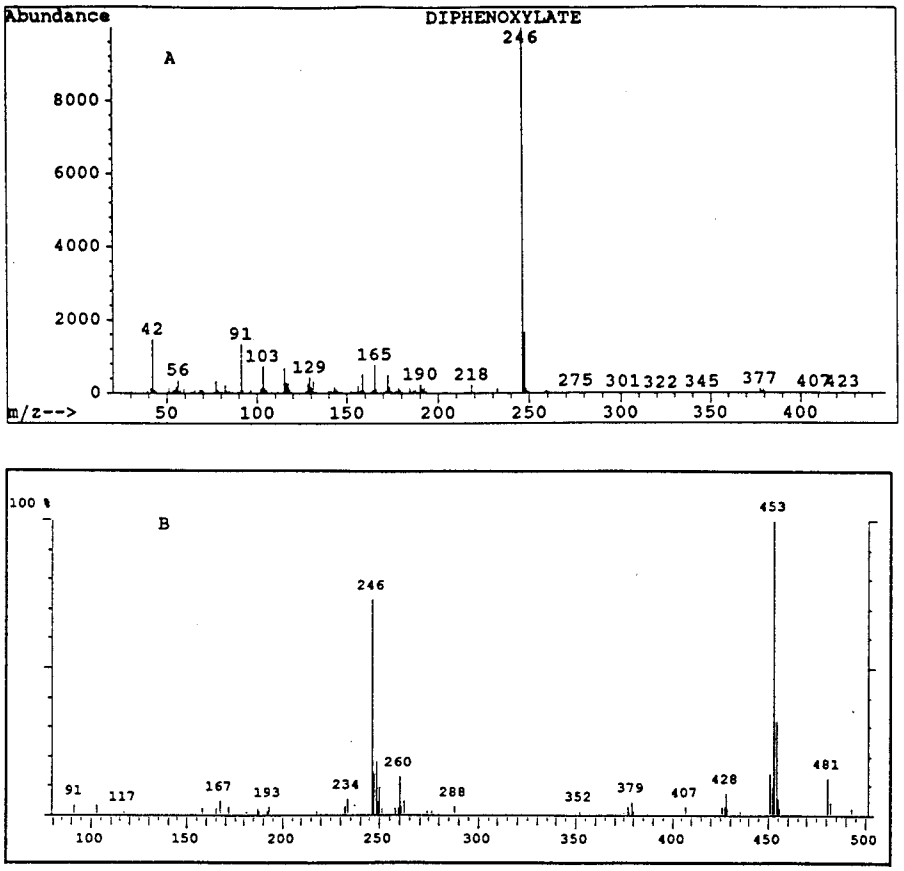

Figure 1. Mass spectra of diphenoxylate: (A) EI, (B) methane PICI.

MD, or Sigma-Aldrich Chemical Co., St. Louis, MO.

A Finnigan Mat GCQ system was used in our study for GC-CIMS. The conditions are as follows: Column: DB-5MS, 30m x 0.25 mm x 0.25 um film thickness, carrier gas: helium at 40 cm/sec constant velocity; injector temperature: 250 °C; oven temperature program: 70 °C (hold 1 min) to 300 °C (hold 10 min) at 20 °C/min; transfer line: 275 °C; ion source: 200 °C; CI reagent gas: methane, positive ion CI mode, full scan range 60 to 500 amu; data acquisition start time: 3 min. The total run time was 22.5 min.

EI spectra were obtained on a Hewlett Packard GC/MSD (5890 series II GC and 5971A mass selective detector) with the same type of column. The oven temperature program was the same as that for GC-CIMS, except that the final hold time was 15 min and the column head pressure was 11 psi. Full scan range was from 29 to 450 amu.

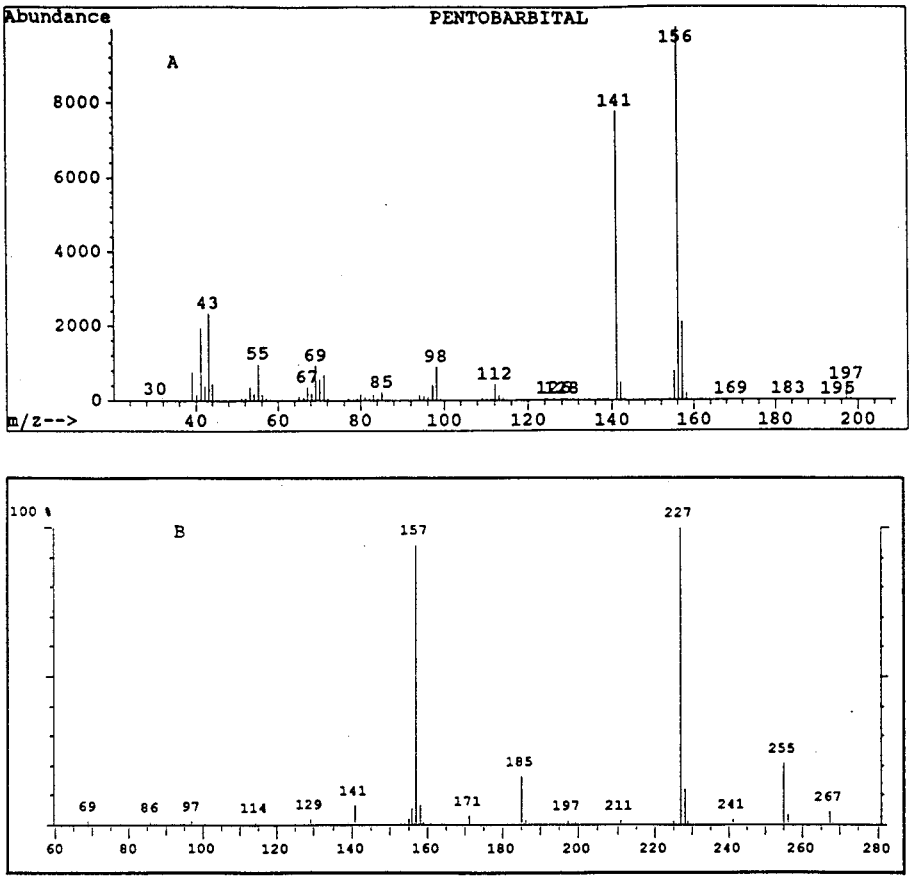

Figure 2. Mass spectra of pentoarbital: (A) EI, (B) methane PICI.

RESULTS AND DISCUSSION

The reconstructed ion current analysis was performed from m/z 60 to 500 and stored in a user created library. Table 1 lists the comparison of the CH_4 CI and EI spectra in the molecular ion region for the 134 compounds tested. In the EI spectra, 52 (39%) gave either no molecular ion (M^+) or <2 % abundance of M^+, and 12 (9%) had between 2 to 5 % abundance of M^+. Only 11 (8%) of the CH_4 CI spectra showed the absence of M + 1 ions. In addition, all standards that produced a protonated molecular ion (MH^+) peak in CH_4 CI also showed a distinctive ion at $[M + 29]^+$, providing further confirmation of the molecular ions (not shown). Representative CIMS spectra are presented in Fig. 1B – 2B.

Under the conditions described above, compounds that usually require HPLC analyses can be easily screened and identified by GC-CIMS. The examples include: amphetamine, chlorpheniramine, diphenhydramine, ethosuximide, haloperidol,

Table 1. Comparison of the EI and methane CI spectra of 134 compounds.

DRUGS	MW	EI M+.	CI MH^+
2-ETHOXYBENZAMIDE	165	Y	Y
ACETAMINOPHEN	151	Y	Y
ALPRAZOLAM	308	Y	Y
AMINOPYRINE	231	Y	Y
AMITRIPTYLINE	277	N	Y
AMPHETAMINE	135	N	Y [1]
ATROPINE	289	Y	Y
BACLOFEN	213	N	Y [2]
BENZTROPINE	307	Y	Y
BERGAPTEN	216	Y	Y
BORNEOL	154	N	Y [1,2]
BROMAZEPAM	315	Y	Y
BROMPHENIRAMINE	318	N	Y
CAFFEINE	194	Y	Y
CAMPHOR	152	Y	Y
CARBAMAZEPINE	236	Y	Y
CARBINOXAMINE	290	N	Y
CARISOPRODOL	260	N	Y
CHLOPHEDIANOL	289	N	Y
CHLORDIAZEPOXIDE	299	Y	Y
CHLOROQUINE	319	Y	Y
CHLORPHENIRAMINE	274	N	Y
CHLORPROMAZINE	318	Y	Y
CHLORPROPAMIDE	276	N	N
CHLORZOXAZONE (3)	169	Y	Y
CLEMASTINE	343	N	Y
CLONAZEPAM	315	Y	Y
CLONIDINE	229	Y	Y
COCAINE	303	Y	Y
CODEINE	299	Y	Y
COUMARIN	146	Y	Y
CYCLANDELATE	276	N	N
CYCLIZINE	266	Y	Y
CYCLOBENZAPRINE	275	N	Y
DEXAMETHASONE (3)	392	N	N
DEXTROMETHORPHAN	271	Y	Y
DIAZEPAM	284	Y	Y
DIHYDROCODEINE	301	Y	Y
DIPHENHYDRAMINE	255	N	Y
DIPHENIDOL	309	N	Y
DIPHENOXYLATE	452	N	Y
DIPYRONE (4)	351	N	N
DOXYLAMINE	270	N	Y
EPHEDRINE	165	N	Y [2]
ESTRADIOL	272	Y	Y
ESTRONE	270	Y	Y
ETHOPROPAZINE	312	N	Y
ETHOSUXIMIDE	141	N	Y
FENFLURAMINE	231	N	Y
FLUOXETINE (3)	309	Y	Y
FLURAZEPAM	387	Y	Y
FURAZOLIDONE (3)	225	Y	Y

DRUGS	MW	EI M+.	CI MH^+
GLUTETHIMIDE	217	Y	Y
GRISEOFULVIN	352	Y	Y
GUAIFENESIN	198	Y	Y
HALOPERIDOL	375	N	Y
HEPTAMINOL	145	N	Y [2]
HEXACHLOROPHENE (3)	406	Y	Y
HOMATROPINE	275	Y	Y
HYDROCODONE	299	Y	Y
IMIPRAMINE	280	Y	Y
LAPACHOL	242	Y	Y
LORATADINE	382	Y	Y
LOVASTATIN	404	N	N
MAZINDOL	284	N	Y [2]
MECLIZINE	390	Y	Y
MEFENAMIC ACID (3)	241	Y	Y
MENTHOL	156	N	Y [1]
MEPHENESIN	182	Y	Y
MEPHENTERMINE	163	N	Y [1]
MESTRANOL	332	Y	Y
METHADONE	309	N	Y
METHAMPHETAMINE	149	N	Y
METHIMAZOLE	114	Y	Y
METHOCARBAMOL	241	N	N
METHYLTESTOSTERONE	302	Y	Y
METOPROLOL	267	N	Y
METRONIDAZOLE	171	Y	Y
MORPHINE (3)	285	Y	Y
NADOLOL	309	N	Y
NALIDIXIC ACID (5)	232	Y	Y
NALORPHINE	311	Y	Y
NAPROXEN (4)	230	Y	Y
NICOTINE	162	Y	Y
NORETHINDRONE	298	Y	Y
NORETHINDRONE ACETATE	340	Y	Y
NORTRIPTYLINE	263	N	Y
ORPHENADRINE	269	N	Y
OXAZEPAM	286	N	Y [2]
OXYCODONE	315	Y	Y
OXYMETHOLONE	332	Y	Y
PENTAZOCINE	285	Y	Y
PENTOBARBITAL	226	N	Y
PHENACETIN	179	Y	Y
PHENAZOPYRIDINE	213	Y	Y
PHENFORMIN (4)	205	N	N
PHENIRAMINE	240	N	Y
PHENMETRAZINE	177	Y	Y
PHENOBARBITAL	232	Y	Y
PHENTERMINE	149	N	Y [1]
PHENYL SALICYLATE (3)	214	Y	Y
PHENYLBUTAZONE	308	Y	Y
PHENYLPROPANOLAMINE	151	N	Y [1,2]

Table 1. (continued).

DRUGS	MW	EI M+.	CI MH^+
PHENYTOIN (4)	252	Y	Y
PREDNISOLONE	360	N	N
PROBENECID (4)	285	Y	Y
PROCAINE	236	N	Y
PROGESTERONE	314	Y	Y
PROMETHAZINE	284	Y	Y
PROPANTHELINE BROMIDE	448	N	N
PROPOXYPHENE	339	N	N
PROPRANOLOL	259	Y	Y
PSEUDOEPHEDRINE	165	N	Y [2]
PULEGONE	152	Y	Y
PYRILAMINE	285	Y	Y
QUINIDINE	324	Y	Y
QUININE	324	N	Y
SANTONIN	246	Y	Y
SECOBARBITAL	238	N	Y

DRUGS	MW	EI M+.	CI MH^+
STANOZOLOL (4)	328	Y	Y
STRYCHNINE	334	Y	Y
SULFADIAZINE (4)	250	N	Y
TESTOSTERONE	288	Y	Y
TESTOSTERONE PROPIONATE	344	Y	Y
TETRAHYDROPALMATINE	355	Y	Y
THEOPHYLLINE	180	Y	Y
THIABENDAZOLE	201	Y	Y
TOLBUTAMIDE	270	N	N
TRIAZOLAM	342	Y	Y
TRIHEXYPHENIDYL	301	N	Y
TRIMETHOBENZAMIDE	388	Y	Y
TRIMETHOPRIM	290	Y	Y
TRIPELENNAMINE	255	Y	Y
TRIPROLIDINE	278	Y	Y

Y = yes, N = no. All standards are prepared at 40 ng/ul in methanol, except as specified.
(1) $[M-H]^+$ (2) MH^+-H2O (3) 80 ng/ul
(4) 200 ng/ul (5) 240 ng/ul.

methamphetamine, nadolol, nortriptyline, oxazepam, phenylpropanolamine, promethazine, pyrilamine, and sulfadiazine.

Aminopyrine and dipyrone, a sulfonate sodium salt of aminopyrine, gave similar CI spectra. However, negative ion chemical ionization can be used to distinguish them. Under the current experimental conditions instead of the MH^+, fragmentation ions are seen for sulfonyl ureas (chlorpropamide and tolbutamide), esters (cyclandelate and propantheline bromide), and for some steroids (prednisolone and dexamethasone). These compounds may be positively identified with further MS-MS experiments using the same instrument.

In this work, we demonstrated the potential of using GC-CIMS as a rapid screening tool for detecting drugs in Chinese Patent Medicines. Compared to the routine GC/MS screening method in the electron impact (EI) process, the results achieved a more conclusive confirmation of drugs. For the 134 compounds tested, 92 % gave the expected molecular weight information in GC-CIMS. Thus, this method enables a rapid and simultaneous detection of 134 drugs/chemicals, and has significant advantages over previously reported GC-MS methods for target compound analysis (Au et al. 2000). The combination of GC and methane chemical ionization can provide mass spectra that withstand legal scrutiny.

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