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HPLC and GC–MS screening of Chinese proprietary medicine for undeclared therapeutic substances

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Abstract

Traditional Chinese medicine includes raw medicinal materials and Chinese proprietary medicine (CPM). Despite being of natural origin, toxic effects, adulteration with synthetic therapeutic substances and even deaths had been associated with CPM. There is thus a need to develop analytical technique to rapidly screen for undeclared toxic and therapeutic substances in CPM. In this study, a high performance liquid chromatography-diode-array detection method was developed and used to screen for undeclared therapeutic substances in CPM. An ultraviolet (UV) library of 266 drugs had been compiled. Solute identification was performed by comparing the analytical data (UV spectra, retention time and relative retention time) with those of the 266 standards. Gas chromatography-mass spectrometry was used as a confirmation method. These chromatographic methods had been shown to be selective and reproducible in screening for undeclared drugs in CPM. Using the method developed, 41 CPM samples in seven categories were screened for undeclared therapeutic substances. One anti-asthmatic CPM was found to contain codeine.

Keywords: Chinese medicine; Reversed phase chromatography; High performance liquid chromatography; Gas chromatographic–mass spectrometry; Codeine

1. Introduction

With the increasing usage of Chinese proprietary medicine (CPM) throughout the world [1], assessment of the safety of CPM becomes an important issue for the health care professions. At present, our knowledge about the constituents of Chinese herbs and their pharmacological and potential toxic effects on humans is extremely limited [2]. Over the past two decades, accidental poisoning by toxic substances in CPM such as aconitine [3], tetrahydropalmatine [4] and various adulterants [5–10] has been previously reported.

Thin layer chromatography (TLC) [11,12] is a simple technique used for detection and identification of undeclared drugs in CPM. The presence of undeclared drug has also been previously studied using high performance liquid chromatography

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Table 1
Range of RT and RRT of 266 standard drugs in the UV library[a]

RT (min)	RRT	Drug name
0–10.00	0.29–1.22	Caffeine, Acetaminophen, Acetazolamide, Amiloride HCl dihydrate, Amino-2,1-(4-nitrophenyl)propane-1,3-diol, Amino-4,6-chlorobenzene-1,3-disulphonamide, Aminophylline, Amodiaquine HCl, Amphetamine Sulfate, Atenolol, Atropine methonitrate, Azathioprine, Barbitone, Barbitone Sodium, Chloroquine diphosphate, Chlorothiazide, Cimetidine, Codeine phosphate, Ephedrine HCl, Flucytosine, Hydrochlorothiazide, Lincomycin HCl, Metformin HCl, Methotrexate, Metronidazole, Minocycline, Morphine HCl, Naloxone, Neostigmine methylsulphate, Nicotinamide, Phenformin HCl, Phenylpropanolamine HCl, Pholcodine, Piracetam, Pseudoephedrine sulphate, Pseudoephedrine HCl, Ranitidine HCl, Resorcinol, Salbutamol, Salbutamol sulphate, Sulfadiazine, Succinylsulphathiazole, Sulfisomidine, Sulphacetamide sodium, Sulphadiazine, Sulphathiazole, Terbutaline sulphate, Theobromine, Theophylline Anhydrous, Tranylcypromine sulphate, Trimethoprim, Vancomycin HCl
10.01–20.00	1.23–2.32	Cefazolin, Thiamphenicol, Lignocaine HCl, Sulfamethazine, Primidone, Sulphamethizole, Sulphadimidine sodium, Chloroquine, Tetracycline HCl, Captopril, Methoxyphenamine, Sulphadimidine, Atropine, Sulphamethoxypyridazine, Pheniramine maleate, Minoxidil, Sulphamethoxydiazine, Sodium salicylate, Strychnine, Carbimazole, Quinidine sulphate, Quinine HCl, Quinidine gluconate, Phenazone, Quinine sulphate, Salicylamide, Metoclopramide HCl, Prednisolone Sodium Phosphate, Aspirin, Guaiphenesin, Timolol maleate, Sulphachloropyridazine sodium, Phthalyl sulphathiazole, Salicylic acid, Sulfadoxine, Spiramycin, Pethidine HCl, Betamethasone sodium phosphate, Antipyrine, Dexamethasone phosphate, Dapsone, Sulphamethoxazole, Phenobarbitone, Pyrimethamine, Phenobarbitone sodium, Chloramphenicol palmitate, Chloramphenicol base, Doxycycline HCl, Sulphafurazole, Papaverine HCl, Noscapine HCl, Chlorthalidone, Trifluprom azine, Quininone, Butobarbitone, Pentazocine HCl, Tetrahydropalmatine, Oxprenolol HCl, Chlorpheniramine, Phenacetin, Chlordiazepoxide HCl, Propranolol HCl, Dexchlorpheniramine, Brompheniramine maleate, Proguanil HCl, Sulphachlorpyrazin sodium, Hydrocortisone micronised, Prednisone, Prazosin HCl, Benzylpenicillin potassium, Sulfaquinoxaline, Sulphadimethoxine, Clopamide, Chlormezanone, Dextromethorphan HBr, Papaverine, Fentanyl citrate, Liothyronine, Flurazepam HCl, Oxymetazoline HCl, Aminoethyl-*N*-2,4-t-butyl-2,6-xylylacetamide, Diphenhydramine, Benzocaine, Droperidol, Erythromycin estolate, Methylprednisolone, Amylobarbitone, Coumarin, Vinblastine sulphate, Phenytoin, Phenytoin sodium, Betamethasone, Phenindamine tartrate, Dexamethasone, Fenfluramine HCl, Nystatin, Promethazine base, Oxazepam, Hydrocortisone-21-hemisuccinate sodium salt, Promazine HCl, Hydrocortisone sodium succinate, Hydrocortisone hydrogen succinate, Quinalbarbitone sodium, Beclomethasone, Lorazepam
20.01–30.00	2.34–3.40	Phenolphthalein, Fluoxymesterone, Frusemide, Fluocinolone acetonide, Imipramine HCl, Thyroxine sodium, Sulfinpyrazone, Berberine HCl, Oestradiol benzoate, Triprolidine HCl, Nortriptyline HCl, Fluocortolone, Thiomersal, Cinchocaine HCl, Prednisolone acetate, Dextropropoxyphene HCl, Perphenazine, Sulindac, Carbamazepine, Fludrocortisone acetate, Verapamil HCl, Prednisone acetate, Aconitine, Terconazole, Haloperidol, Cortisone acetate, Griseofulvin, Temazepam, Ketoprofen, Lormetazepam, Oxyphenbutazone, Naproxen sodium, Diflunisal, Hydrocortisone, Chlorpropamide, Isosorbide dinitrate, Rifampicin-quinone, Piroxicam, Chlorpromazine HCl, Fluphenazine decanoate, Tolbutamide, Clomipramine HCl, Oestradiol, Betamethasone dipropionate, Nandrolone, Dexamethasone acetate, Prochlorperazine mesylate, Bendroflumethiazide, Norethisterone base, Ethinyloestradiol, Propantheline bromide, Warfarin sodium, Testosterone, Beclomethasone-17-propionate, Flunitrazepam, Beclomethasone dipropionate, Thioridazine HCl, Amino-2,5-nitrobenzophenone, Trifluoperazine HCl, Estradiol, Diazepam, Ethisterone, Cyclopenthiazide, Cyproheptadine HCl, Clotrimazole, Stilboestrol, Fluocinonide, Beclomethasone-21-propionate, Niflumic acid, Dexamphetamine sulphate, Methyltestosterone, Indomethacin, Diclofenac, Bumetanide, Norgestrel, Betamethasone-17-valerate, Ibuprofen, Prednisolone pivalate, Nalidixic acid, Prednisolone caproate, Digitoxin, Phenylbutazone, Cinnarizine base, Amino-2,5-chlorobenzophenone, Diphenoxylate HCl
30.01–36.26	3.48 –4.19	Tamoxifen citrate, Mefenamic acid, Spironolactone, Norethisterone acetate, Artemisinin, Fluocortolone-21-pivalate, Stanozolol, Stilboestrol dipropionate, Miconazole nitrate, Sodium fusidate, Fusidic acid sodium, Progesterone, Buclizine HCl, Malathion, Mestranol, Phenoxybenzamine HCl, Norethandrolone, Terfenadine, Hydrocortisone-21-caprylate, Tolnaftate, Hexachlorophene, Testosterone propionate

[a] Caffeine was used as the internal standard.

(HPLC) [13–16]. However, these are not mass screening methods. In addition, the sensitivity and selectivity of gas chromatography–mass spectrometry (GC–MS) [17] is of particular advantage for the identification of undeclared drugs in CPM. Among these methods, the coupling of HPLC to diode-array detector (DAD) has been gaining more importance [18–23]. None of the above reported methods were specifically applied to rapid mass screening for undeclared therapeutic substances in CPM.

Table 2
Main classifications of drugs listed in the library

Classification	Number of drugs present
Analgesics	17
Anticancer	3
Antiinflammatory	12
Antiarrhythmic	3
Antibiotic	38
Anticholinergic	3
Anticonvulsant	5
Antidepressant	4
Antidiabetic	4
Antifungal	7
Antihistamine	13
Antihypertensive	10
Antimalarial	10
Antipsychotic	9
Anxiolytic	6
Beta-adrenoceptor agonist	2
Bronchodilator	7
Cardiovascular agent	4
Cholinergic	1
Corticosteroid	31
Cough Suppressant	5
Disinfectant	2
Diuretic	10
Gastro-intestinal agent	5
Hormone	20
Hypnotic	10
Immunosuppressive	1
Keratolytic	2
Local anaesthetic	3
Pediculicide	1
Stimulant	6
Sympathomimetic	2
Antithyroid	1
Uricosuric, antigout	1
Vitamin	1
Other chemicals	7

In this study, a rapid and specific high performance liquid chromatography-diode-array detector (HPLC-DAD) method was applied as a screening method for undeclared therapeutic substances in CPM. GC–MS method was used as a confirmation method.

2. Experimental

2.1. Materials

All chemicals used were analytical grade or better. Acetonitrile and methanol (HPLC grade) were purchased from Reagent Chemical Industry Ltd. (Thailand). Water for HPLC was treated with a Milli-Q water purification system (Millipore, France). The majority of the standards drugs used were of British Pharmacopoeia (BP), or The United State Pharmacopoeia (USP) standards. Some secondary standard drugs were obtained from the Pharmaceutical Laboratory, Department of Scientific Services, Institute of Science and Forensic Medicine, Singapore.

2.2. High performance liquid chromatography

Hewlett Packard (HP) series 1050 quaternary gradient pump, photo-diode array detector, HP series 1100 autosampler were used. System control, data acquisition and process, and auto-library search were performed using software of HP ChemStation for LC 3D. A HP Lichrosorb reversed phase (RP) C18 200×4.6 mm^2, 10 μm (particle size) column was used.

Gradient elution (acetonitrile/phosphate buffer) was performed as follows: Solvent A = sodium dihydrogen phosphate buffer (25 mM, pH = 3.2); solvent B = acetonitrile; step gradient is from 10% to 30% of B over 10 min, then to 50% of B over another 10 min, and finally to 70% of B over 10 min and maintained for another 5 min. Total chromatography duration was 35 min. The equilibration time between two consecutive injections was set at 5 min (total cycle time 40 min). The flow-rate of mobile phase was 1 ml min^{-1}. Injection volume was 10 μl. The detection wavelengths were set at 220, 254 and 280 nm. The ultraviolet

Table 3
HPLC detection levels (DL), regression equations and coefficients of determination (r^2) of 31 standard drugs at 254 nm (unless otherwise specified)

No.	Drug Name	DL (mg l^{-1})	Regression equation	Coefficient of determination (r^2)
1	Acetazolamide	0.13	$y = 1.520x - 10.790$	0.998
2	Paracetamol	0.17	$y = 1.139x - 6.712$	0.999
3	Strychnine	0.25	$y = 0.774x + 62.330$	0.995
4	Chlorpromazine HCl	0.25	$y = 0.767x + 44.570$	0.994
5	Amodiaquine HCl	0.25	$y = 0.758x - 26.320$	0.997
6	Quinine sulphate	0.31	$y = 0.624x - 24.060$	0.999
7	Naproxen sodium	0.41	$y = 0.471x - 0.093$	1.000
8	Diclofenac sodium	0.42	$y = 0.461x + 0.137$	0.999
9	Berberine	0.67	$y = 0.288x - 2.813$	0.999
10	Dextromethorphan HBr[a]	1.00	$y = 0.192x - 2.378$	0.999
11	Chlorpheniramine	1.83	$y = 0.105x - 8.449$	0.996
12	Naloxone	1.99	$y = 0.097x + 3.952$	0.998
13	Crotonic acid	3.09	$y = 0.062x + 0.104$	1.000
14	Tolbutamide	3.15	$y = 0.061x - 0.458$	0.999
15	Atenolol	3.51	$y = 0.056x - 0.534$	1.000
16	Lincomycin HCl[a]	3.65	$y = 0.053x + 0.735$	0.999
17	Methoxyphenamine	4.91	$y = 0.039x + 0.313$	0.998
18	Emetine	5.24	$y = 0.037x - 0.082$	0.997
19	Primidone	6.33	$y = 0.030x + 0.032$	0.999
20	Ibuprofen	6.96	$y = 0.028x + 0.221$	0.998
21	Lignocaine HCl	7.21	$y = 0.027x - 0.633$	0.996
22	Fenfluramine HCl	8.27	$y = 0.023x + 0.846$	0.999
23	Aconitine	8.51	$y = 0.023x + 2.132$	0.991
24	Ephedrine HCl	8.91	$y = 0.021x + 0.716$	0.994
25	Barbitone	10.90	$y = 0.176\ x + 0.035$	0.999
26	Digitoxin	12.14	$y = 0.016x + 0.196$	0.999
27	Diphenhydramine	12.45	$y = 0.015x + 0.582$	0.984
28	Artemisinine[a]	15.89	$y = 0.012x - 0.049$	0.999
29	Amylobarbitone	19.44	$y = 0.010x - 0.194$	0.999
30	Dextropropoxyphene HCl	22.24	$y = 0.009x + 1.083$	0.971
31	Atropine	30.19	$y = 0.006x + 0.153$	0.997

[a] Detection λ at 220 nm.

(UV) spectra from 200 to 400 nm were recorded on-line during the chromatographic run. Caffeine was used as the internal standard (IS).

2.2.1. Ultraviolet library

Methanol solutions (1 mg ml^{-1}) of 266 drugs (Table 1) were prepared, filtered using 0.45 μm membrane filter and injected into the HPLC system as described. The UV spectra of these 266 standard drugs were obtained using DAD and compiled as an UV library. Solute identification in CPM samples was carried out by library search. Unknown UV data were compared with those in the library. Library matches of UV spectra were automatically calculated for each peak and a score of 1000 represents a perfect match.

2.2.2. Detection level determination

To determine the detection level, 31 drugs of various UV sensitivity were selected. Most determinations were carried out at a wavelength of 254 nm, except for three, which were performed at a wavelength of 220 nm. Four concentrations, ranging from 0.1 to 1.0 mg ml^{-1}, of each standard were used for the calibration curves. The detection level (DL) was determined from the calibration curve by, $DL = 3 \times SD/slope$ of calibration curve [24], where, SD is the standard deviation of

Table 4
Range of RT and RRT of 58 standard drugs using GC–MS[a]

RT (min)	RRT	Drug name
6.09–9.91	0.45–0.73	Fenfluramine HCl, Phenylpropanolamine HCl, Ephedrine HCl, Pseudoephedrine HCl, Methoxyphenamine, Phendimetrazine tartrate, Barbitone, Salicylamide
10.43–19.24	0.77–1.42	Benzocaine, Ibuprofen, Guaiphenesin, Tolbutamide, Caffeine, Dimenhydrinate, Carisoprodol, Diphenhydramine, Lignocaine, Chlorpheniramine, Minoxidil, Mefenamic acid, Theophylline Anhydrous, Ketoprofen, Diclofenac, Propranolol HCl, Oxymetazoline HCl, Atropine, Imipramine HCl, Pentazocine HCl, Promethazine base, Bromhexine HCl, Phenylbutazone, Codeine phosphate, Lorazepam, Diazepam, Morphine HCl, Chlorpromazine HCl
20.38–29.27	1.52–2.17	Trimethoprim, Metoclopramide HCl, Cinchonine, Methyltestosterone, Ethinyloestradiol, Norethandrolone, Griseofulvin, Progesterone, Bisacodyl, Testosterone propionate, Indomethacin, Prednisolone, Dexamethasone, Fluoxymesterone, Haloperidol, Tetrahydropalmatine, Prochlorperazine mesylate, Miconazole nitrate, Cinnarizine base, Buclizine HCl, Strychnine, Diphenoxylate HCl

[a] Caffeine was used as the internal standard.

the blank (methanol) response which can be described as the standard deviation of the noise value of 12 blank injections.

2.3. Gas chromatography–mass spectrometry

HP 6890 series of GC system fitted with HP 6890 series injection and HP 6890 series mass selective detector were used in this analysis. The analytes were separated with a HP-5 MS capillary column (5% phenyl-95% methyl siloxane; 25 m × 0.2 mm internal diameter capillary) with the carrier gas (helium) set at 1 ml min^{-1}. A 1.0 μl volume of the sample was injected using the splitless mode. The data acquisition system was controlled by MS ChemStation. Full scan mass spectra were collected between 50 and 550 amu at 1.53 scan s^{-1}. The MS was operated in the electrospray ionization mode. The initial oven temperature was set at 80°C. It was then increased to 300°C at 10°C min^{-1}. The final temperature of 300°C was held for 10 min. The total running time was 32 min. The injection volume was 1 μl. The Wiley standard chemical MS library [25] and spectra of reference standards were used in the drug identification.

To determine the detection levels (DL), nine drugs (Chlorpheniramine, Diphenhydramine, Diazepam, Codeine, Barbitone, Fenfluramine, Atropine, Caffeine, and Dexamethasone) were selected randomly. Different concentrations of these drugs in methanol were prepared and injected into the GC–MS. The DL were determined by visual evaluation [24].

2.4. Sample preparation

Forty one CPM samples were purchased from a Chinese medical hall, the Chinese Proprietary Medicine and Medical Liquor Centre, in Singapore. The sample preparation procedures were as follows:

(a) For capsules, tablets, pills or powder: 20 ml of ethanol (95% denatured) were added to 1 g of

Table 5
DL of nine standard drugs under the GC–MS method

Drug name	DL (mg l^{-1})
Diazepam	1.35
Chlopheniramine	2.24
Diphenhydramine	3.80
Codeine	4.00
Barbitone	7.20
Fenfluramine	18.00
Atropine	20.00
Caffeine	28.00
Dexamethasone	116.00

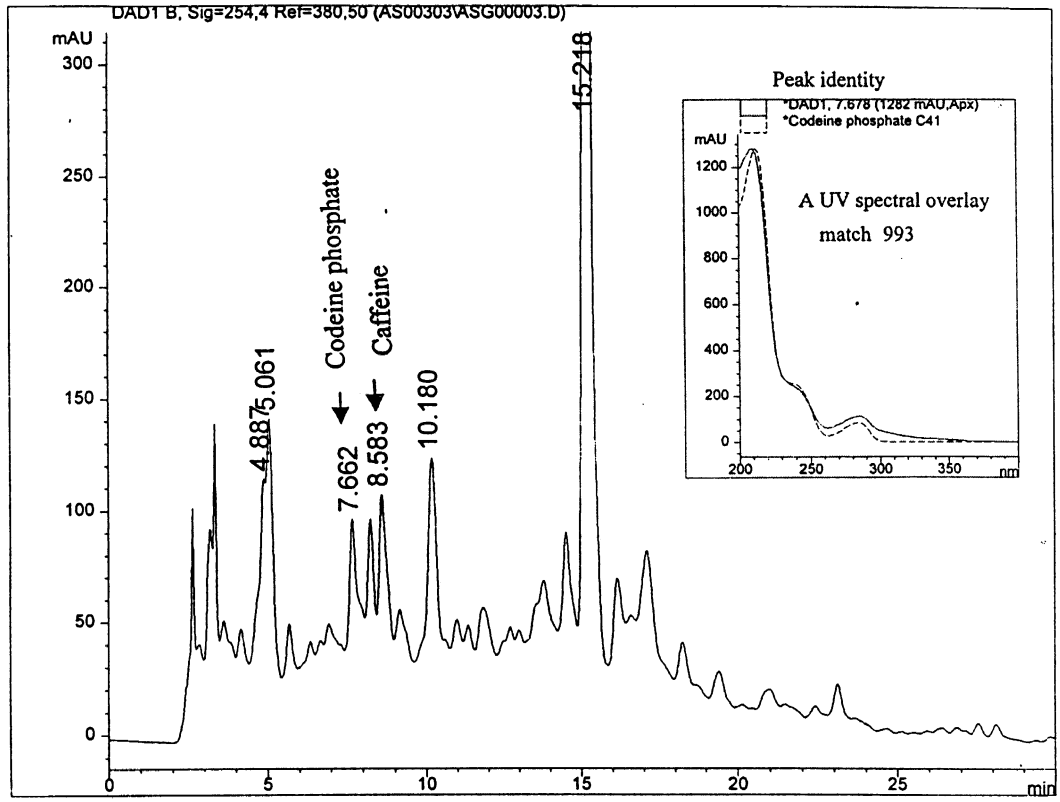

Fig. 1. Detection of codeine phosphate in CPM AsthmaWan by HPLC showing chromatogram and UV profile of codeine phosphate.

finely ground CPM samples. These mixtures were then heated to boiling and filtered. This was re-peated 3 times. The filtrates were collected and evaporated to dryness by rotary evaporator. The residue was dissolved in 4 ml of methanol and filtered by 0.45 μm membrane filter for HPLC and GC–MS analysis.

(b) For syrup and liquid: 20 ml of ethanol (95% denatured) were added to 3 g of CPM samples. It was then heated to boiling. After centrifugation (4000 rpm, 5 min), the supernatants were decanted and evaporated to near dryness by rotary evaporator. The residue was dissolved in 4 ml of methanol and filtered by 0.45 μm membrane filter for HPLC and GC–MS analysis.

3. Results

3.1. Development of a fast screening method by high performance liquid chromatography

A library of the UV spectra of 266 standard drugs was created. Table 1 showed the drug name, retention time (RT) and relative retention time (RRT) of the 266 drugs in this UV library. Table 2 showed the distribution of drugs according to the therapeutic categories. Drugs in the UV library were from various therapeutic classes.

The inter-day variations of the RT and RRT of 266 drugs were investigated. Relative standard deviation (RSD) range of RT was from

0.0% to 17.6%. RT of the majority of drugs (75%) were found to have RSD $\leqslant$ 5%. The RSD of the RRT of all drugs was good (RSD $\leqslant$ 5%). These variations were attributed mainly to the accumulation of residual matter in the column. Extensive flushing with methanol and/or tetrahydrofuran returned the RT to smaller variations.

CPM extracts containing 35 spiked drugs were screened by HPLC. All of the spiked standard drugs were successfully detected and identified by the described HPLC screening method (library matches more than 970, the highest library match is 1000). DL of 31 standards were between 0.13 and 30.19 mg l^{-1}. Among them, 28 determinations were performed at 254 nm, and three standards that have no absorption at 254 nm were determined at 220 nm. The DL, regression equations and coefficients of determination were shown in Table 3. The most sensitive drug is acetazolamide with DL of 0.13 mg l^{-1}. The

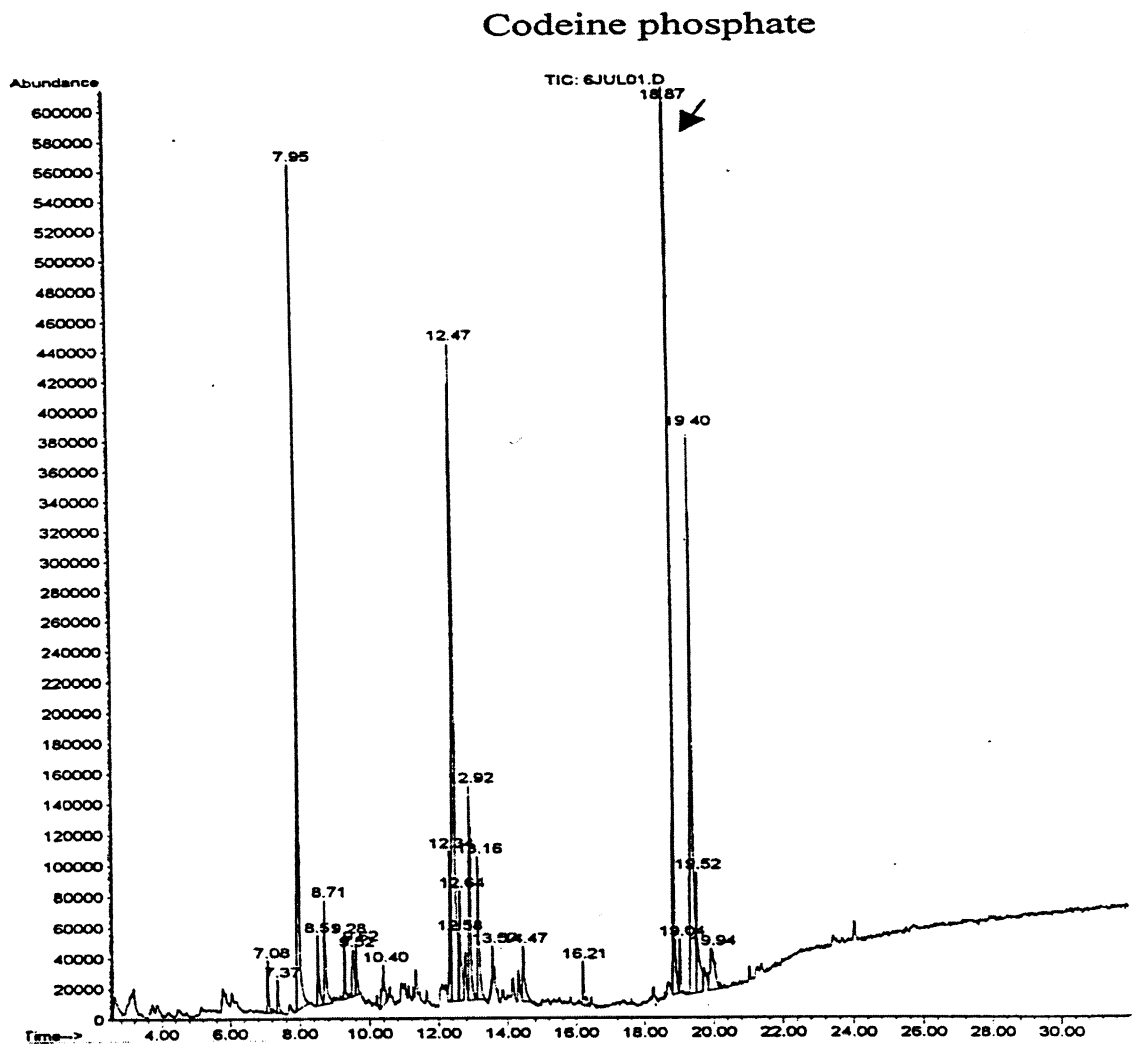

Fig. 2. Detection of codeine phosphate in CPM AsthmaWan by GC–MS showing a total ion chromatogram (TIC) and mass spectrum of codeine phosphate.

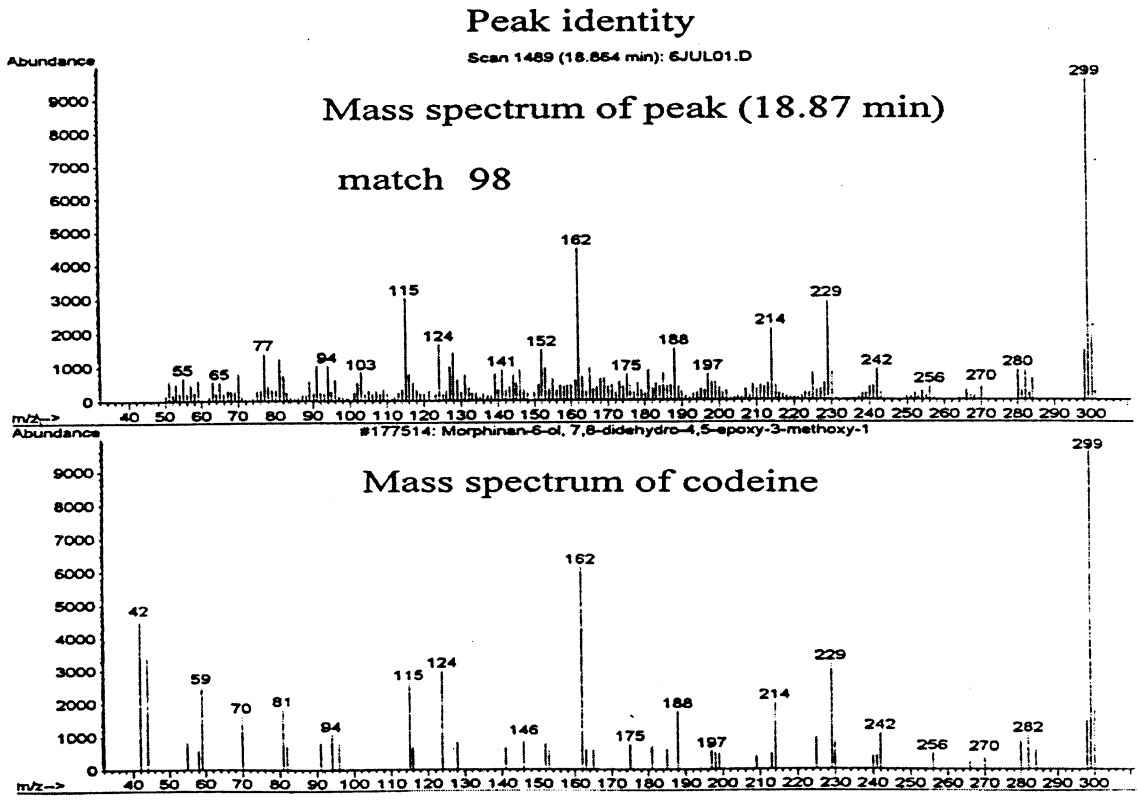

Fig. 2. (*Continued*)

highest DL determined is for atropine (30.19 mg l^{-1}). Most of the DL of these 31 drugs (80%) falls within the range of 0.1–10 mg l^{-1}.

3.2. Gas chromatography-mass spectrometry determination

The RT and RRT of 58 drugs were compiled in Table 4. These 58 standards could be eluted with the current method and identified directly using the MS library search without derivatization.

DL of 9 drugs (chosen randomly) were determined and shown in Table 5. Most of these drugs could be detected at a level between 1.0 and 30.0 mg l^{-1}. The most sensitive drug was diazepam with DL = 1.4 mg l^{-1}. The highest DL determined was for dexamethasone (116.0 mg l^{-1}).

4. Discussion

4.1. Screening for undeclared therapeutic substances in Chinese proprietary medicine

The large number of potential undeclared therapeutic drugs and sometimes their low concentration level in herbal matrix had made the analysis difficult. There was absolutely no information about the potential undeclared drugs present in the CPM before analysis. HPLC-DAD is especially useful in the screening for undeclared drugs in CPM. To the authors' knowledge, this is the first report of HPLC-DAD method developed for rapid mass screening for undeclared and/or toxic therapeutic substances in CPM.

4.2. Peak identification

Coelution of two or more compounds remains one of the major causes of errors in chromatographic analysis. Therefore, it is absolutely necessary to check the peak of interest for purity. This can be done automatically by HP ChemStation software.

In the current study, the majority of the RT of the spiked standards differed from the library data by $\leqslant 10\%$. This agrees well with a previous report, which quoted 15% [26]. Therefore only those compounds, which have been identified by library search (UV spectra comparison) and with less than 15% difference in their RT compared to that of corresponding library data, would be considered. The RRT is also considered in drug identification.

The UV library match indicated how closely the unknown spectrum matched the library data. Match of 1000 indicated identical spectra and below 900 indicated a different one [26]. A peak identification result with a library match above 950 could be considered as identification with good certainty.

4.3. Ultraviolet library

A UV absorbance spectrum of a compound might depend on the physical and chemical characteristics of the solvent in which the compound is dissolved. For HPLC-DAD, this means that the spectrum might be dependent on the mobile phase. Therefore, it is strongly recommended [26] that an UV spectral library should be dedicated to a single HPLC-DAD analysis method. In this study, all standard drugs and samples were analysed by the same HPLC method. RT, RRT and UV spectrum of each standard drug was obtained under this HPLC method and stored into the library.

4.4. Sensitivity

The sensitivity of the HPLC-DAD method varied from compound to compound. In previous reports [19,26], the sensitivity of most drugs was about 0.1 mg l^{-1}, some standards could be detected at 5–60 μg l^{-1}. In this study, the DL of the standards selected range from 0.13 to 30.19 mg l^{-1} (Table 3). Some of these values were much higher than those found in the reference reports. The reason is that the method developed is a general screening procedure for 266 drugs. It is not optimized for individual drugs.

4.5. Gas chromatography–mass spectrometry determination

The drug identification was performed by both library search using the commercially available Wiley MS library and testing pure reference standards in the GC–MS determination. Although the number of standard drugs which could be detected under the current GC–MS method was small (Table 4), they could be identified whenever they were present.

5. Application

Forty one CPM samples in seven categories were purchased from a Chinese medical shop, of which 25 CPM are anti-asthmatic preparations. The 41 CPM extracts were screened by the HPLC and GC–MS methods developed.

One anti-asthmatic CPM, blister packed green capsules of AsthmaWan (Yangcheng brand from China), was found to contain codeine by both HPLC (Fig. 1) and GC–MS (Fig. 2) methods. The amount of codeine in AsthmaWan was found to be 61.8 μg/capsule [27]. Eleven herbal ingredients were stated on the product package while nine were stated on the product insert [27], none of which will give rise to codeine. Codeine is one of the opioid alkaloids found in species of the *Papaveraceae* family [28]. It can be used as a cough suppressant (dosage 45–120 mg/day) and analgesic (dosage 120–240 mg/day) [29]. According to the Medicines Act [30,31] of Singapore, the presence of codeine in CPM is not allowed. It had been suggested [22] that presence of drugs detected from screening must systematically be confirmed by a GC–MS determination. In this study, GC–MS was used as a confirmation method.

6. Conclusion

CPM often contains complex herbal matrices. The screening and identification of undeclared therapeutic substances in CPM is a very challenging task. Using a combination of UV profiles, RT and RRT, a simple, rapid and specific HPLC-DAD method was developed. GC–MS was used as a further confirmation method. These chromatographic systems have shown to be selective and reproducible. Using the method developed, out of 41 CPM samples, one CPM was found to contain undeclared codeine. Greater awareness of and control over the safety and quality of CPM are necessary.

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