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Application of a computer-assisted high-performance liquid chromatographic multi-wavelength ultraviolet detection system to simultaneous toxicological drug analyses

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SUMMARY

An emergency drug screening system for the separation and identification of toxic drugs, MULTI-HPLC, is presented. Chromatographic peaks, which were impossible to identify with a conventional high-performance liquid chromatographic UV detection system, became distinguishable by the spectral search and retention prediction of the data-processing program MCASYST. Sixty-five toxic drugs, frequently identified in drug poisonings in Japan, were selected as references in the drug library. Retention time, optimum detection wavelength, detection limit and recoveries from serum and urine were listed. Possible applications of the system are demonstrated, using gastric contents, sera and urines in cases of multiple drug ingestion. Quantitative analysis was sufficiently sensitive and precise to permit clinical diagnosis with increased accuracy.

INTRODUCTION

The rapid and accurate analysis of the cause of a poisoning case is difficult and requires a competent, intensive investigator, especially in cases of multiple drug ingestion[1,2]. However, recent progress in liquid chromatography may permit the automation of a systematic procedure[3–5]. We report here a newly developed, sophisticated drug-screening system, MULTI-HPLC, a reversed-phase high-performance liquid chromatographic (HPLC) assay for toxic drugs in biological samples by multi-wavelength UV detection and an automated identification system, and its clinical applications.

Sixty-five toxic drugs, frequently identified in drug poisoning cases in Japan[6], were selected as reference drugs; these should cover *ca.* 95% of drug poisoning cases. According to their pharmacological effect, they were classified as antipsychoactive, antianxiety, anticonvulsant and antidepressant drugs. They were also classified accor-

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ding to their structures as barbiturates, benzodiazepines, butyrophenones and phenothiazines. In the MULTI-HPLC system these drugs, which have various physico-chemical properties, can be analysed simultaneously with a single sample injection.

EXPERIMENTAL

Liquid chromatography

The HPLC system consisted of a Model 880-PU pump and a Multi-320 multi-channel UV detector (Jasco, Tokyo, Japan). The column was a Jasco FineSil $C_{18}S$ (250 mm × 4.6 mm I.D.) maintained at 50°C. The mobile phases were mixtures of 10 m*M* perchloric acid, 10 m*M* sodium perchlorate and acetonitrile and the flow-rate was 1 ml/ min.

The data handling was performed with a NEC9801Vm_2 personal computer and data processor (DP-L320, Jasco), whose functions include data acquisition by the multi-channel UV detector, data processing, generation of three-dimensional chromatograms and contour chromatograms and peak deconvolution. A microcomputer-assisted separation system (MCASYST) was also used with the MULTI-HPLC system. The components of MCASYST are illustrated schematically in Fig. 1.

Spectrum search function. The multi-wavelength UV detector yields a specific absorption spectrum for each drug. Even when some drugs show similar or identical retention times, comparison of spectra with reference spectra, provided by a stored database drug library, helps to identify the compounds.

Retention prediction program. This is based on the statistically and experimentally determined equation

$$\log k' = (-0.0178X + 1.2003)\log P_e - (0.0034X + 0.4562)$$

where X is the volume fraction of the organic solvent, k' is the capacity factor of the solute, and P_e is a physico-chemical parameter of the solute. If X and log k' are

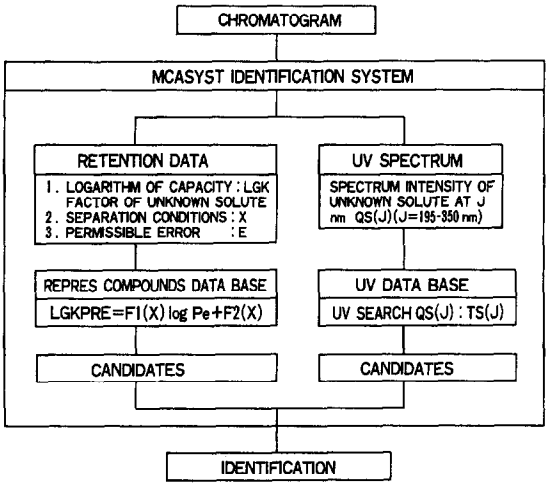

Fig. 1. Algorithm of automated identification in MCASYST.

determined, then log P_e for a toxic substance can be predicted and its identity searched for in the stored data file[5].

Materials

All pure drugs used were kindly donated by Shionogi (Osaka, Japan), Fujisawa (Osaka, Japan), Sankyo (Tokyo, Japan), Roche (Tokyo, Japan), Upjohn (Tokyo, Japan), Takeda (Osaka, Japan) and Yoshitomi (Osaka, Japan). The standard samples were dissolved in acetonitrile at a concentration of 100 μg/ml.

Human body fluids, such as gastric contents, sera and urines from poisoned patients, were collected at the Critical Care Medical Centre (CCMC), Nippon Medical School, Tokyo, Japan. Volumes of 100 μl of biological samples and 200 μl of acetonitrile were shaken and then centrifuged at 9600 *g* for 20 min. After centrifugation, the extracts were passed through an Ekikurodisk cartridge (Gelman Science, Tokyo, Japan).

RESULTS AND DISCUSSION

Recovery

To test the efficiency and reproducibility of the extraction procedure, absolute recoveries from plasma and urine samples were determined. The recoveries of the 65 drugs registered in the standard drug library are listed in Table I. Drugs, that have a low solubility in acetonitrile could not be quantitatively recovered from serum.

Optimum detection wavelength

The spectra of the 65 drugs obtained with the multi-wavelength UV detector were similar to those measured by a double-beam UV spectrometer. The optimum detection wavelength was at the maximum UV absorption of the spectrum of each drug.

Linearity

A linear response with the multi-wavelength UV detector at the optimum detection wavelength of each drug was obtained except some poorly soluble drugs. The relationship between concentration and peak area was linear up to 500 μg/ml for the 65 standard drugs.

Detection limit

The detection limit was dependent on the quality and age of the column used. Limits of detection based on a signal-to-noise ratio of 3:1 and using new, high-quality columns are listed in Table I.

Clinical applications

Possible applications of the system to gastric contents, sera and urines in drug poisoning cases were demonstrated. In drug poisoning cases the patients had sustained depression or schizophrenia and received psychiatric treatment. In suicidal attempts the patients had ingested large amounts of prescribed drugs. No information about the toxic compounds taken by the patients was available at the time of the HPLC analysis, because the patients were often in a comatose state on arrival at the CCMC.

TABLE I

LIST OF SUBSTANCES REGISTERED IN STANDARD DRUG LIBRARY

The 65 toxic drugs most frequently encoutered in Japan are listed. Approximately 95% of drug poisoning cases should be covered.

Common name	*Retention time (min)*	*k'*	*Log k'*	*Log P_e*	*Maximum detection wavelength (nm)*	*Detection limit (ng)*	*Recovery (%)*	
							Urine	*Serum*
Acetaminophen	2.49	0.556	−0.255	0.480	245	2.81	142	100
Caffeine	2.87	0.794	−0.100	0.713	210	1.48	176	100
Barbital	2.91	0.819	−0.087	0.734	210	3.95	135	100
Sulpiride	2.87	0.794	−0.100	0.713	215	1.84	98	100
Acetylsalicylic acid	4.14	1.588	0.201	1.168	230	4.59	31	33
Phenobarbital	4.57	1.856	0.269	1.271	210	3.21	76	76
Bromvalerylurea	4.71	1.944	0.289	1.301	210	7.63	92	96
Ethenzamide	5.02	2.138	0.330	1.364	210	1.39	107	101
Bromazepam	4.61	1.881	0.274	1.280	235	4.50	113	101
Phenacetin	5.45	2.406	0.381	1.442	245	3.41	82	82
Cloxazolam	4.79	1.994	0.300	1.318	240	3.52	77	40
Oxazolam	5.14	2.213	0.345	1.386	240	4.02	95	63
Chlormezanone	6.41	3.006	0.478	1.588	230	4.89	49	52
Chlordiazepoxide	5.72	2.575	0.411	1.486	245	3.52	101	101
Pentobarbital	7.22	3.513	0.546	1.690	210	11.25	90	76
Nitrazepam	7.03	3.394	0.531	1.667	275	5.36	89	105
Amobarbital	7.45	3.656	0.563	1.716	210	8.49	91	91
Phenytoin	7.45	3.656	0.563	1.716	210	4.79	57	56
Secobarbital	8.91	4.569	0.660	1.863	210	13.64	91	150
Carbamazepine	8.76	4.475	0.651	1.849	215	3.31	103	116
Glutethimide	8.99	4.619	0.665	1.870	210	4.89	96	96
Oxazepam	8.45	4.906	0.691	1.909	230	5.36	102	99
Nimetazepam	11.34	6.088	0.784	2.051	265	9.00	98	87
Estazolam	11.33	6.081	0.784	2.050	225	10.23	80	90
Diazepam	11.92	6.450	0.810	2.089	240	52.63	110	110
Flunitrazepam	14.11	7.819	0.893	2.215	220	11.54	131	133
Flurazepam	13.06	7.163	0.855	2.158	230	11.84	98	96
Alprazolam	15.02	8.388	0.924	2.261	225	72.00	83	83
Medazepam	12.56	6.850	0.836	2.129	255	8.65	84	88

Haloperidol	14.95	8.344	0.921	2.258	210	14.52	96	92
Propericyazine	14.69	8.181	0.913	2.245	270	10.00	106	109
Triazolam	19.67	11.294	1.053	2.457	220	8.33	83	90
Bromperidol	16.91	9.569	0.981	2.348	210	20.45	68	68
Promethazine	16.37	9.231	0.965	2.324	250	9.68	42	44
Desipramine	17.64	10.025	1.001	2.379	210	9.78	96	96
Carpipramine	13.84	7.650	0.884	2.201	210	25.71	114	142
Maprotyline	21.14	12.213	1.087	2.508	210	11.84	91	95
Nortriptyline	20.91	12.069	1.082	2.500	210	6.92	95	100
Hydroxyzine	20.80	12.000	1.079	2.497	210	21.43	80	80
Imipraine	20.64	11.900	1.076	2.491	210	15.00	100	100
Trihexyphenidyl	22.49	13.056	1.116	2.552	210	50.00	93	80
Amitriptyline	24.90	14.563	1.163	2.624	210	21.43	103	107
Trimipramine	27.30	16.063	1.206	2.688	210	26.87	105	96
Levomepromazine	26.11	15.319	1.185	2.657	250	21.43	107	107
Clocapramine	24.88	14.550	1.163	2.623	210	40.00	109	109
Chlorpromazine	32.76	19.475	1.289	2.815	255	22.50	19	9
Clomipramine	38.26	22.913	1.360	2.921	210	40.00	94	94
Primidone	3.25	1.031	0.013	0.885	210	3.69	80	83
Trimethadione	3.64	1.275	0.106	1.025	210	23.08	80	1
Haloxazolam	4.49	1.806	0.257	1.253	245	3.88	72	31
Metharbital	4.57	1.856	0.269	1.271	220	7.14	82	91
Mephobarbital	7.95	3.969	0.599	1.770	210	6.52	115	140
Clofedanol	9.18	4.738	0.676	1.886	210	7.50	95	92
Acetylpheneturide	10.72	5.700	0.756	2.008	210	9.00	40	42
Clonazepam	11.14	5.963	0.775	2.037	210	5.23	113	115
Fludiazepam	19.61	11.256	1.051	2.455	235	10.23	121	118
Zotepine	37.69	22.556	1.353	2.911	215	19.15	139	136
Mianserine	11.49	6.181	0.791	2.061	210	6.92	118	113
Clotiazepam	12.56	6.850	0.836	2.129	215	9.78	97	102
Perphenazine	17.02	9.638	0.984	2.353	255	23.08	100	100
Timiperone	9.33	4.831	0.684	1.899	245	3.85	91	86
Etizolam	20.02	11.513	1.061	2.469	210	10.98	96	87
Biperiden	18.26	10.413	1.018	2.403	210	31.58	110	96
Bromocriptine	29.41	17.381	1.240	2.740	210	46.15	74	74
Indomethacin	52.72	31.950	1.504	3.140	210	27.69	86	93

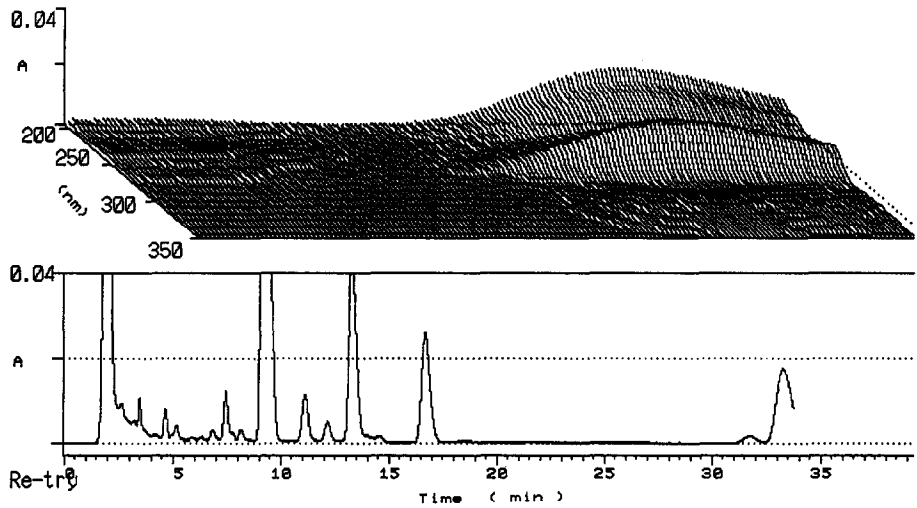

Fig. 2. Real-time display in case 1.

Using the functions of the MCASYST, the analytical procedures for identifing the unknown peaks in a sample chromatogram were the same as described previously[7]. The chromatogram of the extract of gastric contents in case 1 is shown in Figs. 2 and 3. Fig. 2 shows a real-time display during MULTI-HPLC analysis. The analyst can obtain a three-dimensional chromatogram of the unknown sample at the time of the analysis. Fig. 3A is a three-dimensional chromatogram, Fig. 3B is a contour map and Fig. 3C is a conventional chromatogram, obtained at 225 nm. Fig. 4 shows the printed output for peak 6 (at 16.71 min) in case 1. The UV search assigned it to promethazine with a coefficient of 0.99 (the definition is given in previous papers[3,4]). Fig. 5 shows the UV spectra for peak 6. The upper spectrum in the measured one and the lower spectrum that of the promethazine standard in the UV spectral database. The retention search also indicated this compound as the first candidate with a coefficient[5] of 0.837. The final result from both searches in the system shown in Table II indicated that promethazine was the best candidate.

The results from the MCASYST system, including coefficients for retention prediction (RPC) and spectral search (SSC), concentrations, and the lists of drugs prescribed, are shown in Tables II–IV for cases 1–3, respectively. In cases 1–3, more than six drugs in gastric contents were identified by the MCASYST system.

The results for serum and urine in case 3 are also shown in Table IV. Possible applications of the MCASYST system were obtained using biological samples. Gastric samples are most useful in determining the kind of drugs that have been taken by a patient. It is difficult to distinguish the compounds in the spectral search of serum and urine samples, as they may have been metabolized. However, there appears to be no interference with peak identification from common serum or urine components.

Recently, cases of "polydrug overdose" or "multiple drug ingestion" have increased[8,9]. We have been making strong efforts to analyse these cases by conventional HPLC–UV systems, whereas rapid and accurate analysis by the MULTI-HPLC system is useful for "first-aid" treatment of poisoning in emergencies. However, in-

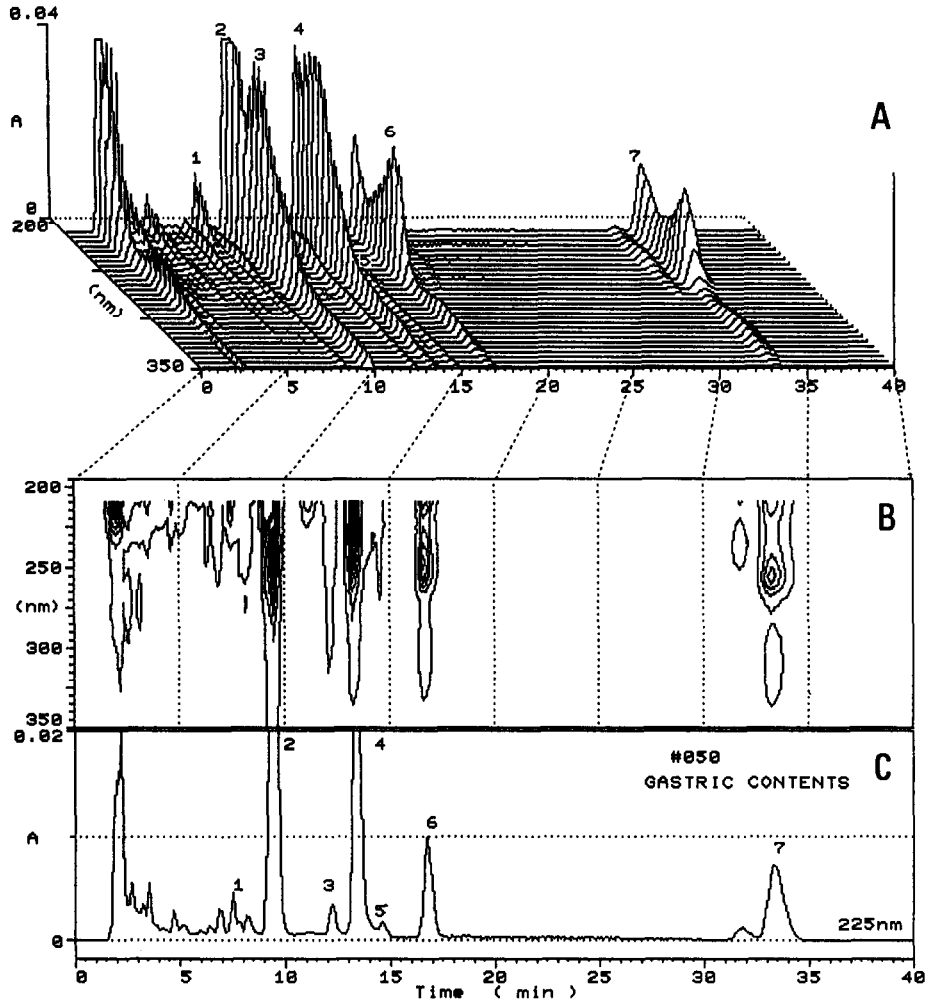

Fig. 3. (A) Three-dimensional chromatogram, (B) contour plot map chromatogram and (C) chromatogram at 225 nm for case 1. Peaks: 1 = pentobarbital; 2 = glutethimide; 3 = diazepam; 4 = flurazepam; 5 = flunitrazepam; 6 = promethazine; 7 = chlorpromazine.

formation that was obtained from the patients about toxic compounds after their recovery from a comatose state was dubious in the most poisoning cases. The patients often said that they had injested "the prescribed drugs", which were not known in detail. Not only the number but also the name of the tablets taken remained uncertain. In clinical scenes, it should not be necessary for information obtained from patients to be required for scientific confirmation, and indeed it would be dangerous. In the cases examined here the results are different from the lists of prescribed drugs, which means that other drugs must have been taken in addition to the prescribed drugs. In some cases the patient must have ingested a few kinds of drugs and not all the drugs that had been prescribed. The possibility that the drugs ingested by the

PEAK No.6 T_R=16.71

UV SPECTRAL SEARCH

No.	COMPOUND	CORRELATION
1	PROMETHAZINE	0.99
2	ACETAMINOPHEN	0.95
3	PHENACETIN	0.95
4	LEVOMEPROMAZINE	0.93
5	HALOXAZOLAM	0.93

RETENTION SEARCH (ERROR<20%)

No.	COMPOUND	log Pe	CORRELATION
1	PROMETHAZINE	2.324	0.837
2	ALPRAZOLAM	2.261	0.688
3	HALOPERIDOL	2.258	0.663
4	BROMPERIDOL	2.348	0.647
5	PERFENAZINE	2.354	0.595

SYSTEM OUTPUT		CORRELATION	BARGRAPH
1	PROMETHAZINE	0.913	*********

Fig. 4. MCASYST output of spectral search and retention prediction for peak 6 in case 1.

patients are different from those prescribed is demonstrated in the text. The results by the MULTI-HPLC system were also confirmed by thin-layer chromatography in each case.

Although the MULTI-HPLC system in its present form leaves many problems to be solved in practice, the concept has opened up a new dimension in emergency toxicology.

TABLE II

RESULTS OF PEAK IDENTIFICATION AND QUANTITATIVE ANALYSIS OF GASTRIC CONTENTS IN CASE 1

Case 1 = 55-year-old female, schizophrenia. RPC = retention prediction coefficient; SSC = spectral search coefficient.

Drugs prescribed	*Drug measured*	*RPC*	*SSC*	*Concentration (μg/ml)*
Lofepramine	Pentobarbital	0.992	0.99	70.62
Maprotyline	Glutethimide	0.974	0.99	820.63
Haloperidol	Diazepam	0.880	0.99	11.55
Cloxazolam	Flunitrazepam	0.896	0.98	6.23
Biperiden	Flurazepam	0.818	0.99	216.33
Haloxazolam	Promethazine	0.837	0.99	256.26
Flurazepam	Chlorpromazine	0.770	0.99	159.89
Pentobarbital				
Glutethimide				
Chlorpromazine				
Promethazine				

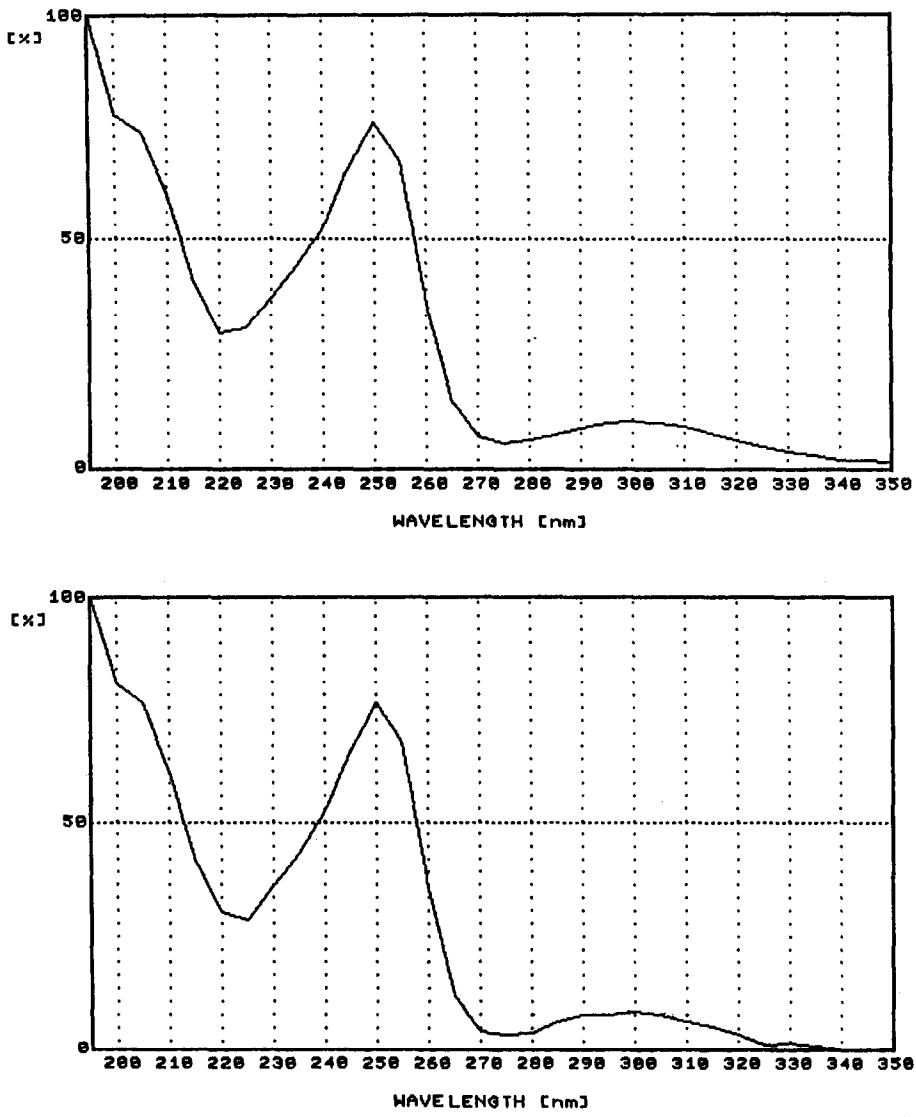

Fig. 5. (Top) spectrum of the compound in peak 6 and (bottom) spectrum of promethazine in the spectral library.

CONCLUSIONS

The computer-assisted MULTI-HPLC drug-screening system with simultaneous, multi-wavelength UV detection was developed for automated identification of toxic drugs. Chromatographic peaks that were impossible to identify by conventional HPLC became distinguishable by the spectral analysis and retention predicition of the data-processing program MCASYST and the accuracy of identification was satis-

TABLE III

RESULTS OF PEAK IDENTIFICATION AND QUANTITATIVE ANALYSIS OF GASTRIC CONTENTS IN CASE 2

Case 2 = 34-year-old male, depression. RPC = retention prediction coefficient; SSC = spectral search coefficient.

Drugs prescribed	*Drugs measured*	*RPC*	*SSC*	*Concentration (μg/ml)*
Sulpiride	Acetylsalicylic acid	0.840	0.96	345.76
Cloxazolam	Phenobarbital	0.738	0.99	1549.78
Trihexyphenidyl	Ethenzamide	0.997	0.95	893.62
Diazepam	Diazepam	0.932	0.99	217.42
Phenobarbital	Promethazine	0.837	0.99	797.84
Promethazine	Hydroxyzine	0.914	0.95	34.38
Chlorpromazine	Chlorpromazine	0.827	0.99	754.65
Estazolam				
Haloxazolam				

TABLE IV

RESULTS OF PEAK IDENTIFICATION AND QUANTITATIVE ANALYSIS OF GASTRIC CONTENTS, SERUM AND URINE IN CASE 3

Case 3 = 57-year-old male, depression. RPC = retention prediction coefficient; SSC = spectral search coefficient.

Drugs prescribed	*Drugs measured*	*RPC*	*SSC*	*Concentration (μg/ml)*
Ergotamine tartrate	*Gastric contents*			
Phenobarbital	Phenobarbital	0.629	0.99	19.10
Estazolam	Nitrazepam	0.644	0.99	246.7
Hydrochlorothiazide	Amitriptyline	0.730	0.99	296.9
Dipyridamole	*Serum*			
	Phenobarbital	0.843	0.99	11.15
	Nitrazepam	0.857	–	1.08
	Urine			
	Phenobarbital	0.911	0.99	21.13
	Nitrazepam	0.912	0.91	0.21
	Amitriptyline	0.927	–	1.48

factory. Using the computerized procedure, analysis is speedy and almost automatically processed, avoiding "trial-and-error" condition searches. The samples can be analysed after a single injection into the MULTI-HPLC system. Quantitative analysis proved to be very sensitive and precise, permitting clinical diagnosis with increased accuracy. The MULTI-HPLC drug screening system is considered to be clinically practical and beneficial.

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