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Reversed-phase high-performance liquid chromatographic database of retention indices and UV spectra of toxicologically relevant substances and its interlaboratory use

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Abstract

An HPLC identification system based on a 1-nitroalkane retention index scale and secondary retention index standards is described. The retention index values and spectral data for 383 toxicologically relevant compounds (therapeutic and illicit drugs, environmental toxins and endogenous compounds) are given. The retention data may be directly applied in any laboratory using any reversed-phase, base-deactivated column, provided that the standardized procedure is observed.

1. Introduction

The identification of toxic compounds in biological material presents a challenging task for an analytical toxicologist and is of the utmost importance in clinical toxicology wards and in forensic institutes. From the clinical point of view, the identification of a toxic substance, which always precedes a proper determination, may influence the therapeutic means applied in the case of acute poisoning. On the other hand, the forensic relevance of the correct and unequivocal identification of a poison is obvious.

Toxicologists have developed several methodical approaches that are particularly useful for the identification of toxic substances, under the common name of "systematic toxicological analysis" (STA). A method applicable for STA should meet the following requirements: it should cover a broad range of relevant substances in one analytical run; it should be universally applicable and the data should be exchangeable between any laboratories; and it should use several uncorrelated identification parameters.

The STA Committee of the International Association of Forensic Toxicologists (TIAFT) has adapted and optimized several identification procedures that may fulfil the above requirements. So far chromatographic methods such as thin-layer chromatography (TLC) and gas chromatography (GC) have appeared especially useful for the identification of unknown toxic substances. TLC and GC procedures have been standardized and comprehensive, multi-laboratory databases for about 6000 compounds have been established [1–3]. Particularly valuable is the combination of mass spectrometry with standardized GC retention parameters, applied after standardized sample preparation [4].

High-performance liquid chromatography (HPLC), applied as an identification method, shows similar sensitivity and selectivity to GC.

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The advent of the diode-array detector brought a potentially very powerful identification system, combining retention parameters and UV spectra. It appeared, however, that the nominally identical column packings of various commercial origin show strikingly different selectivities and these differences were difficult to compensate through standardization. Therefore, a number of toxicologists have developed their own databases, but the exchange of data was hardly feasible owing to the use of non-standardized retention parameters [5–15].

In previous studies, we have developed a standardized HPLC identification system, based on retention parameters and UV spectra, recorded by means of a diode-array detector. The retention and spectral data for 225 toxicologically relevant substances have been published [16]. The retention was expressed on a 1-nitroalkane retention index scale, using selected drugs as secondary retention index standards. The evolution of this concept and its final form have been described elsewhere [17–22]. This system allows comparable results to be obtained using different reversed-phase columns, also among different laboratories [23,24].

The purpose of this study was to establish a database of toxicologically relevant substances (therapeutic and illicit drugs, pesticides and endogenous compounds), which may be used in various toxicological laboratories using different reversed-phase (octyl- or octadecylsilica) columns and the same, standardized procedure. The database can be expanded in the future, and the data may be exchanged.

2. Experimental

2.1. Standard substances

A range of 365 toxicologically relevant substances, obtained from various manufacturers in pure form, were diluted in acetonitrile–water (1:1) to a concentration of 0.1 mg/ml. Ten benzodiazepine derivatives were subjected to acid hydrolysis and extracted according to Schütz [25]. The hydrolysis products obtained were diluted in acetonitrile–water (1:1) to a concentration corresponding to 0.1 mg/ml of parent drug. The retention and spectral data of all pure substances were stored in the HPLC library.

2.2. Biological extracts

Serum and autopsy blood and liver samples were checked for the absence of drugs and extracted with diethyl ether at pH 4.6 or with dichlormethane–2-propanol (9:1) at pH 8.5, with and without an acid hydrolysis step. Retention and spectral data of three peaks found in acidic extracts and of five peaks found in basic extracts were included in the HPLC database. The detailed conditions and results of these experiments have been described elsewhere [26].

2.3. Chromatographic conditions and equipment

Gradient elution with a mixture of acetonitrile and triethylammonium phosphate buffer (25 m*M*, pH 3.0), as in our previous studies [16,24], was applied. Triethylammonium phosphate buffer (1 *M*, pH 3.0) was supplied by Fluka (Buchs, Switzerland) and diluted with HPLC-grade water before use. The gradient profile was 0–70% acetonitrile in 30 min and 5 min at 70% acetonitrile. The flow-rate was 1 ml/min and the injection volume was 5–10 μl. The equilibration time between two consecutive injections was 10 min.

All experiments were performed using a low-pressure gradient system, consisting of a Type L 6200 gradient pump, AS 2000 autosampler (both from Merck, Darmstadt, Germany) and a Type 990 diode-array detector (Waters, Eschborn, Germany). Three Superspher 100 RP-18, 4-μm particle size, fully end-capped columns (125 × 4 mm I.D.) were supplied by Merck. These columns were used throughout the study.

2.4. Standardization procedure and parameters collected

Retention times and UV spectra of all substances were stored as primary identification parameters. As secondary retention parameters,

Table 1
Retention indices of secondary retention index markers

Acidic mixture		Basic mixture	
Compound	*I*	Compound	*I*
Paracetamol	234	Morphine	198
Barbital	287	Chloroquine	265
Brallobarbital	359	Benzoylecgonine	295
Pentobarbital	405	Cocaine	336
Secobarbital	437	Diphenhydramine	385
Clobazam	484	Haloperidol	409
Indomethacine	610	Amitriptyline	446
Prazepam	648	Thioridazine	504
		Meclozine	601
		Amiodarone	762

the retention indices of all compounds were calculated from their retention times by linear interpolation between standard drugs, whose retention indices had been previously determined on the 1-nitroalkane scale [16]. Separate sets of standard drugs were used for acidic/neutral and basic drugs (Table 1, Figs. 1 and 2).

Standard drug mixtures were examined with every series of compounds examined. As a rule, the retention times of these standards were very stable for the same column. The calculated retention index values were stored in the library together with corresponding retention times and UV spectra. The HPLC software of the Type 990 detector allows a compound to be identified by means of the UV spectrum and selected retention parameter (retention time or retention index). All measurements were performed in duplicate, on different days.

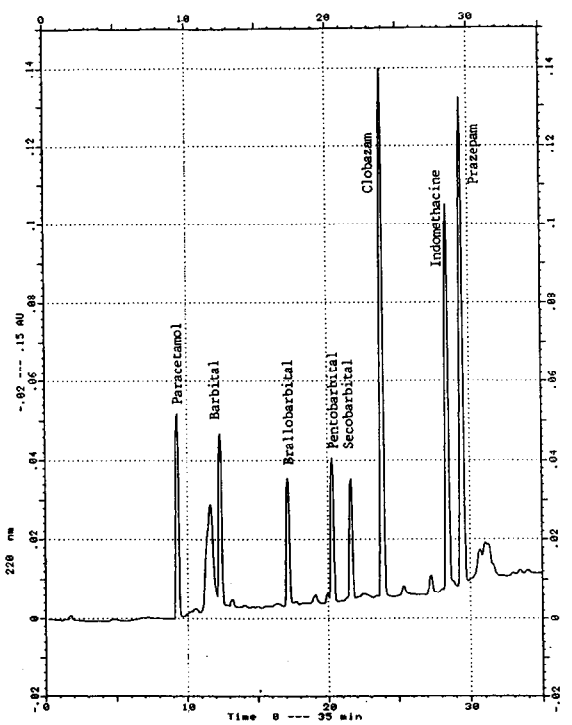

Fig. 1. Chromatograms of secondary retention index standards (acidic/neutral mixture).

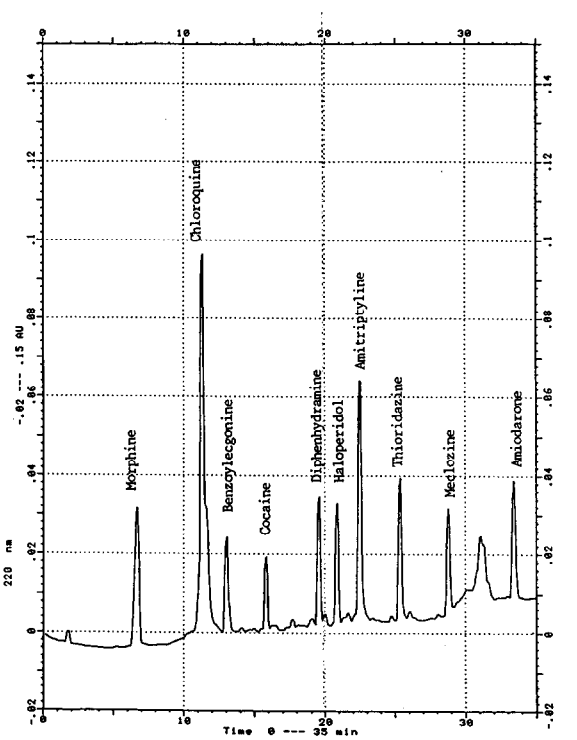

Fig. 2. Chromatograms of secondary retention index standards (basic mixture).

3. Results

Tables 2 and 3 give the data for all 383 substances examined. The absorbance values (A_1^1) given an insight into the prospective detectability of substances by means of a spectrophotometric detector. These data, and UV maxima, are given only for substances showing distinct absorption maxima under the applied conditions. Several compounds (*e.g.*, barbiturates) show only gradually decreasing UV absorbance. For these compounds no absorbance data are given. The last column gives information on whether the retention index values were calculated using acidic/neutral or basic drugs as secondary retention index standards. The collection of I values follows a normal distribution, showing the majority of results in the region between 300 and 500, *i.e.*, in a time span of *ca.* 13 min.

In a previous study [16], we examined the I values of 225 compounds under the same con-

Table 2
Substances examined, in alphabetical order, with their I values, UV maxima, A_1^1 values and scale used

No.	Substance	I	UV	A_1^1	Scale	No.	Substance	I	UV	A_1^1	Scale
1	ABP, 1st peak	461	327	0	ACN	37	Benzoylecgonine	295	274	30	BAS
2	ABP, 2nd peak	495	237	0	ACN	38	Benztropine	461	0	0	BAS
3	ACB	673	236	310	ACN	39	Betaxolol	371	275	76	BAS
4	Acebutolol	311	233	655	BAS	40	Bisacodyl	483	264	270	ACN
5	Acecarbromal	422	0	0	ACN	41	Brallobarbital	359	0	0	ACN
6	Acepromazine	399	278	570	BAS	42	Bromazepam	378	317	65	ACN
7	Acetanilide	316	239	815	ACN	43	Bromisoval	354	0	0	ACN
8	Acetazolamide	249	264	475	ACN	44	Bromophos-ethyl	800	0	0	ACN
9	ADB	704	233	0	ACN	45	Bromoxynil	464	255	380	ACN
10	Ajmaline	328	287	90	BAS	46	Brompheniramine	355	263	250	BAS
11	Alimemazine	437	252	0	BAS	47	Buflomedil	324	276	460	BAS
12	Alimemazine sulphoxide	334	338	0	BAS	48	Bupivacaine	355	0	0	BAS
13	Allobarbital	340	0	0	ACN	49	Bupranolol	373	276	78	BAS
14	Allopurinol	190	251	563	ACN	50	Buprenorphine	386	288	30	BAS
15	Alprazolam	443	276	938	ACN	51	Buspirone	353	238	552	BAS
16	Alprenolol	381	270	69	ACN	52	Butalbital	380	0	0	ACN
17	Amiloride	233	363	800	BAS	53	Butaperazine	496	278	540	BAS
18	Aminophenazone	243	257	443	ACN	54	Butobarbital	365	0	0	ACN
19	Amiodarone	762	240	1200	BAS	55	Caffeine	265	272	504	BAS
20	Amitriptyline	446	239	500	BAS	56	Camazepam	566	314	80	ACN
21	Amobarbital	415	0	0	ACN	57	Carazolol	354	332	438	BAS
22	Amoxapine	372	299	236	BAS	58	Carbamazepine	380	284	490	BAS
23	Amphetamine	241	257	14	BAS	59	Carbaryl	452	280	351	ACN
24	ANB	549	365	0	ACN	60	Carbromal	400	0	0	ACN
25	ANCB	580	356	0	ACN	61	Carbutamide	369	267	149	ACN
26	ANFB	559	357	0	ACN	62	Carpipramine	399	251	184	BAS
27	Aprobarbital	347	0	0	ACN	63	CCB	999	237	0	ACN
28	Ascorbic acid	90	244	556	ACN	64	CCMK	900	271	0	ACN
29	Aspirin	326	274	68	ACN	65	Chlordiazepoxide	357	306	316	ACN
30	Atenolol	224	275	48	BAS	66	Chloridazone	324	285	420	ACN
31	Atrazine	451	264	180	ACN	67	Chlormezanone	382	227	25	BAS
32	Atropine	287	259	17	BAS	68	Chloroquine	265	342	625	BAS
33	Barbital	287	0	0	ACN	69	8-Chlorotheophylline	295	267	604	BAS
34	Benperidol	371	278	230	BAS	70	Chlorpromazine	466	254	1030	BAS
35	Benzocaine	380	284	930	BAS	71	Chlorprothixene	476	267	420	BAS
36	Benzoctamine	370	271	57	BAS	72	Chlorpyrifos	745	288	155	ACN

Table 2 *(continued)*

No.	Substance	I	UV	A_1^1	Scale	No.	Substance	I	UV	A_1^1	Scale
73	Chlorthalidone	359	275	58	ACN	125	Doxylamine	274	263	335	BAS
74	Cimetidine	229	0	0	BAS	126	Droperidol	369	278	190	BAS
75	Cinchocaine	430	327	190	BAS	127	Edifenphos	660	0	0	ACN
76	Clobazam	484	293	75	ACN	128	Ephedrine	224	0	0	BAS
77	Clomethiazole	369	251	270	BAS	129	Estazolam	424	0	0	ACN
78	Clomipramine	471	254	250	BAS	130	Etenzamide	350	293	220	ACN
79	Clonazepam	451	309	350	ACN	131	Ethacrynic acid	516	276	100	ACN
80	Clonidine	237	269	20	BAS	132	Ethosuximide	284	0	0	ACN
81	Clopamide	356	240	346	ACN	133	Ethylmorphine	283	282	48	BAS
82	Clopenthixol	456	270	370	BAS	134	Etilefrine	183	273	100	BAS
83	Clorazepate	464	311	55	ACN	135	Etomidate	466	237	450	ACN
84	Clotiapine	397	299	174	BAS	136	Euphylline	270	271	399	BAS
85	Clotiazepam	551	243	580	ACN	137	Fenbufen	522	285	835	ACN
86	Clozapine	330	294	650	BAS	138	Fenethylline	325	273	240	BAS
87	Cocaine	336	275	30	BAS	139	Fenfluramine	364	264	22	BAS
88	Codeine	243	284	55	BAS	140	Fenoprofen	587	0	0	ACN
89	Colchicine	357	351	420	BAS	141	Fentanyl	377	0	0	BAS
90	Coumarin	368	315	607	ACN	142	Flecainide	410	298	294	BAS
91	Cyclobarbital	374	0	0	ACN	143	Fluanisone	398	244	550	BAS
92	Cyclopentobarbital	377	0	0	ACN	144	Flumazenil	362	245	914	ACN
93	DCFB	458	236	0	ACN	145	Flunarizine	571	252	599	BAS
94	Demeton-S-methyl	402	0	0	ACN	146	Flunitrazepam	459	309	330	ACN
95	Demeton-S-methylsulphone	660	0	0	ACN	147	Flunitrazepam, 3-hydroxy	418	311	0	ACN
96	Demoxepam	388	308	600	ACN	148	Flunitrazepam, 7-amino	336	236	0	ACN
97	Desalkylflurazepam	459	230	0	ACN	149	Flunitrazepam, nor	424	312	0	ACN
98	Desipramine	421	251	308	BAS	150	Fluoxetine	450	263	130	BAS
99	Dexchlopheniramine	342	263	0	BAS	151	Flupenthixol	487	267	294	BAS
100	Dextromethorphan	370	281	70	BAS	152	Fluphenazine	480	307	85	BAS
101	Dextromoramide	440	0	0	BAS	153	Flurazepam	392	309	50	ACN
102	Dextropropoxyphene	438	255	12	BAS	154	Fluvoxamine	412	252	340	BAS
103	1,4-Diaminobutane	80	0	0	BAS	155	Furosemide	414	275	600	ACN
104	1,5-Diaminopentane	90	0	0	BAS	156	Glafenine	322	345	467	ACN
105	Diamorphine	327	276	46	BAS	157	Glibenclamide	623	302	63	ACN
106	Diazepam	529	229	1100	ACN	158	Gliclazide	538	261	20	ACN
107	Diazinon	687	247	35	ACN	159	Glipizide	464	275	230	ACN
108	Diazoxide	345	264	436	ACN	160	Gliquidone	723	311	50	ACN
109	Dibenzepin	349	0	0	BAS	161	Glisoxepide	453	230	70	ACN
110	Diclofenac	630	275	320	ACN	162	Gluthetimide	430	0	0	ACN
111	Digoxin	389	0	0	BAS	163	Guaifenesine	307	275	140	ACN
112	Dihydrocodeine	237	281	47	BAS	164	Haloperidol	409	245	340	BAS
113	Dilthiazem	392	238	661	BAS	165	Harmane	301	299	0	BAS
114	Dimenhydrinate	100	276	268	BAS	166	Heptabarbital	404	0	0	ACN
115	Dimethindene	334	260	613	BAS	167	Hexobarbital	404	0	0	ACN
116	Dimethoate	336	0	0	ACN	168	Hydrochlorothiazide	275	270	640	ACN
117	Diphenhydramine	385	254	17	BAS	169	Hydrocodone	262	282	50	BAS
118	Dipyridamole	387	284	650	BAS	170	Hydromorphone	231	282	50	BAS
119	Disopyramide	327	261	195	BAS	171	Hydroxyzine	428	230	420	BAS
120	Disulfiram	730	279	680	BAS	172	Hyoscine butyl bromide	338	0	0	BAS
121	Disulfoton	752	0	0	ACN	173	Ibuprofen	650	0	0	ACN
122	Diuron	468	250	900	ACN	174	Idobutal	395	0	0	ACN
123	Dothiepin	428	260	260	BAS	175	Imipramine	434	251	300	BAS
124	Doxepin	401	294	230	BAS	176	Indole	448	270	0	BAS

(Continued on p. 102)

Table 2 *(continued)*

No.	Substance	I	UV	A_1^1	Scale	No.	Substance	I	UV	A_1^1	Scale
177	Indomethacin	610	317	180	ACN	230	Midazolam	386	250	360	ACN
178	Isoniazid	132	264	390	ACN	231	Minaprine	278	266	392	BAS
179	Isosorbide dinitrate	445	0	0	BAS	232	MNFB, 1st peak	525	230	0	ACN
180	Ketamine	294	270	25	BAS	233	MNFB, 2nd peak	630	231	0	ACN
181	Ketazolam	583	242	500	ACN	234	Molsidomine	272	296	318	BAS
182	Ketobemidone	295	273	80	BAS	235	6-Monoacetylmorphine	276	283	50	BAS
183	Ketotifene	365	299	480	BAS	236	Monolinuron	455	246	1130	ACN
184	Labetalol	350	303	100	BAS	237	Moperon	387	245	564	BAS
185	Levomepromazine	446	305	140	BAS	238	Morazone	344	261	200	BAS
186	Levorphanol	308	281	80	BAS	239	Morphine	198	285	50	BAS
187	Lidocaine	285	0	0	BAS	240	Nadolol	271	269	40	BAS
188	Linuron	559	249	900	ACN	241	Naftidrofuryl	460	282	141	BAS
189	Loprazolam	379	330	900	ACN	242	Nalorphine	237	284	50	BAS
190	Lorazepam	422	230	1100	ACN	243	Naloxone	251	282	40	BAS
191	Lormetazepam	474	229	1030	ACN	244	Naproxene	476	262	200	ACN
192	Loxapine	399	296	184	BAS	245	Nicotinamide	146	261	450	ACN
193	LSD	358	315	0	BAS	246	Nicotine	100	260	200	BAS
194	MACB	826	236	0	ACN	247	Nifedipine	503	275	0	ACN
195	MAFB	339	235	0	ACN	248	Niflumic acid	586	288	800	ACN
196	Matrotiline	440	0	0	BAS	249	Nikethamide	280	260	280	ACN
197	MATRAC1	379	0	0	ACN	250	Nimodipine	641	237	0	ACN
198	MATRAC2	720	279	0	ACN	251	Nitrazepam	430	311	364	ACN
199	MATRAC3	404	323	0	ACN	252	Nitrendipine	610	236	1340	ACN
200	MATRBAS1	520	279	0	BAS	253	Nitrofurantoin	307	366	770	ACN
201	MATRBAS2	226	0	0	BAS	254	Nitroglycerine	531	0	0	ACN
202	MATRBAS3	344	258	0	BAS	255	Nizatidine	221	320	748	BAS
203	MATRBAS4	384	260	0	BAS	256	Nomifensine	346	237	250	BAS
204	MATRBAS5	270	279	0	BAS	257	Nordiazepam	464	230	1200	ACN
205	MDA	261	285	200	BAS	258	Norephedrine	190	0	0	BAS
206	MDB	750	265	0	ACN	259	Norfenefrine	80	273	171	BAS
207	MDE	244	285	200	BAS	260	Normethadone	417	0	0	BAS
208	MDMA	280	275	215	BAS	261	Norpethidine	339	0	0	BAS
209	Meclozine	601	228	390	BAS	262	Nortriptyline	418	239	898	BAS
210	Medazepam	395	254	860	ACN	263	Noscapine	354	312	85	BAS
211	Melperone	327	248	415	BAS	264	Opipramol	387	254	870	BAS
212	Mephenytoin	382	0	0	ACN	265	Orciprenaline	180	278	140	BAS
213	Mepivacaine	300	0	0	BAS	266	Orphenadrine	416	0	0	BAS
214	Mescaline	255	269	35	BAS	267	Oxazepam	441	228	1200	ACN
215	Metamizol	289	0	0	ACN	268	Oxazolam	340	240	698	ACN
216	Metapramine	358	270	181	BAS	269	Oxprenolol	332	0	0	BAS
217	Methadon	443	0	0	BAS	270	Oxycodone	260	279	40	BAS
218	Methamphetamine	255	255	15	BAS	271	Oxydemeton-S-methyl	269	0	0	ACN
219	Methaqualone	455	266	350	BAS	272	Oxyphenbutazone	503	237	1400	ACN
220	Methohexital	503	0	0	ACN	273	Papaverine	346	249	1800	BAS
221	Methomyl	270	234	0	ACN	274	Paracetamol	234	243	680	ACN
222	Methyl phenidate	316	0	0	BAS	275	Paraoxon	479	270	249	ACN
223	Methylphenobarbital	420	0	0	ACN	276	Parathion-methyl	621	275	238	ACN
224	Metipranolol	363	278	40	BAS	277	Paroxetin	385	294	0	BAS
225	Metoclopramide	308	309	420	BAS	278	Pecazine	440	252	900	BAS
226	Metolachlor	618	0	0	ACN	279	Pemoline	281	0	0	BAS
227	Metoprolol	317	273	50	BAS	280	Penfluridol	716	270	50	BAS
228	Metronidazole	236	320	520	ACN	281	Pentazocine	357	278	70	BAS
229	Mianserine	390	278	75	BAS	282	Pentobarbital	405	0	0	ACN

Table 2 *(continued)*

No.	Substance	*I*	UV	A_1^1	Scale	No.	Substance	*I*	UV	A_1^1	Scale
283	Pentoxifylline	320	275	314	BAS	334	Spironolactone	594	287	570	ACN
284	Perazine	418	252	900	BAS	335	Strychnine	292	254	370	BAS
285	Periciazine	405	269	780	BAS	336	Sulphadiazine	260	267	140	ACN
286	Perphenazine	438	255	800	BAS	337	Sulpiride	240	291	70	BAS
287	Pethidine	334	0	0	BAS	338	Sulthiam	321	246	390	ACN
288	Phenacemide	312	0	0	ACN	339	Suprofen	156	273	0	ACN
289	Phenacetin	356	246	650	ACN	340	Tebuconazole	607	0	0	ACN
290	Phenazone	303	256	450	ACN	341	Temazepam	466	228	1100	ACN
291	Phencyclidine	281	0	0	BAS	342	Terbutryn	494	260	370	ACN
292	Phenelzine	377	0	0	BAS	343	Terfenadine	567	0	0	BAS
293	Pheniramine	279	0	0	BAS	344	Tetracaine	381	314	600	BAS
294	Phenobarbital	357	0	0	ACN	345	Tetrazepam	538	228	1100	ACN
295	Phenprocoumon	609	310	450	ACN	346	Tetroxoprim	286	272	113	BAS
296	Phentermine	277	0	0	BAS	347	Δ^9-THC	999	279	40	ACN
297	Phenylbutazone	651	237	450	ACN	348	Thebaine	324	285	250	BAS
298	1-Phenylethylamine	199	257	0	BAS	349	Theobromine	224	272	560	BAS
299	2-Phenylethylamine	198	257	0	BAS	350	Theophylline	239	270	540	BAS
300	Phenytoin	415	0	0	ACN	351	Thiabendazole	283	302	1120	ACN
301	Pindolol	277	263	300	BAS	352	Thiopental	481	285	790	ACN
302	Pipamperone	286	248	315	BAS	353	Thioridazine	504	261	1000	BAS
303	Piracetam	178	0	0	ACN	354	Tiaprofenic acid	475	306	570	ACN
304	Piribedil	301	286	203	BAS	355	Tilidine	337	0	0	BAS
305	Piritramid	395	0	0	BAS	356	Timolol	297	297	279	BAS
306	Piroxicam	425	363	420	ACN	357	Tocainide	251	0	0	ACN
307	Prajmaline	390	246	198	BAS	358	Tolbutamide	470	228	581	ACN
308	Prazepam	648	227	1800	ACN	359	Tramadol	314	270	70	BAS
309	Primidone	308	0	0	ACN	360	Trancylpromine	241	261	20	BAS
310	Probenecid	560	249	319	ACN	361	Trazodon	358	249	320	BAS
311	Procainamide	202	279	700	ACN	362	Triamterene	291	359	860	BAS
312	Procaine	236	291	950	BAS	363	Triazolam	452	220	1100	ACN
313	Prochlorperazine	462	257	880	BAS	364	Trichlorfon	800	0	0	ACN
314	Promazine	418	252	1060	BAS	365	Trichlormethiazide	377	267	466	ACN
315	Promethazine	411	249	1050	BAS	366	Trifluoperazine	491	255	750	BAS
316	Propafenone	408	304	107	BAS	367	Trifluperidol 1	350	257	308	BAS
317	Propoxur 1	540	264	84	ACN	368	Trifluperidol 2	436	246	0	BAS
318	Propoxur 2	624	264	84	ACN	369	Triflupromazine	505	257	992	BAS
319	Propanolol	370	290	220	BAS	370	Trifluralin	761	274	248	ACN
320	Propyphenazone	422	272	400	ACN	371	Trimethoprim	345	270	220	ACN
321	Protriptyline	424	290	530	BAS	372	Trimipramine	451	249	300	BAS
322	Proxibarbal	255	0	0	ACN	373	Tripelenamine	337	311	250	BAS
323	Quinidine	316	245	950	BAS	374	Tripolidine	360	280	292	BAS
324	Quinine	308	246	950	BAS	375	Tryptamine	233	278	350	BAS
325	Ranitidine	240	317	500	BAS	376	Tyramine	124	275	110	BAS
326	Reserpine	473	294	200	BAS	377	Verapamil	454	278	130	BAS
327	Saccharin	254	269	80	ACN	378	Viloxazine	321	272	90	BAS
328	Salicylamide	305	299	270	ACN	379	Vinylbarbital	410	0	0	ACN
329	Salicylic acid	331	299	270	ACN	380	Warfarin	555	305	450	ACN
330	Scopolamine	288	0	0	BAS	381	Yohimbine	333	273	220	BAS
331	Secutobarbital	365	0	0	ACN	382	Zolpidem	343	296	482	BAS
332	Secobarbital	437	0	0	ACN	383	Zopiclon	314	304	177	BAS
333	Sotalol	273	270	16	BAS						

Table 3
Substances examined, in order of elution, with their I values, UV maxima, A_1^1 values and scale used

No.	Substance	I	UV	A_1^1	Scale	No.	Substance	I	UV	A_1^1	Scale
1	Norfenefrine	80	273	171	BAS	53	Hydrocodone	262	282	50	BAS
2	1,4-Diaminobutane	80	0	0	BAS	54	Caffeine	265	272	504	BAS
3	Ascorbic acid	90	244	556	ACN	55	Chloroquine	265	342	625	BAS
4	1,5-Diaminopentane	90	0	0	BAS	56	Oxydemeton-S-methyl	269	0	0	ACN
5	Nicotine	100	260	200	BAS	57	Methomyl	270	234	0	ACN
6	Dimenhydrinate	100	276	268	BAS	58	Euphylline	270	271	399	BAS
7	Tyramine	124	275	110	BAS	59	MATRBAS5	270	279	0	BAS
8	Isoniazid	132	264	390	ACN	60	Nadolol	271	269	40	BAS
9	Nicotinamide	146	261	450	ACN	61	Molsidomine	272	296	318	BAS
10	Suprofen	156	273	0	ACN	62	Sotalol	273	270	16	BAS
11	Piracetam	178	0	0	ACN	63	Doxylamine	274	263	335	BAS
12	Orciprenaline	180	278	140	BAS	64	Hydrochlorothiazide	275	270	640	ACN
13	Etilefrine	183	273	100	BAS	65	6-Monoacetylmorphine	276	283	50	BAS
14	Norephedrine	190	0	0	BAS	66	Phentermine	277	0	0	BAS
15	Allopurinol	190	251	563	ACN	67	Pindolol	277	263	300	BAS
16	2-Phenylethylamine	198	257	0	BAS	68	Minaprine	278	266	392	BAS
17	Morphine	198	285	50	BAS	69	Pheniramine	279	0	0	BAS
18	1-Phenylethylamine	199	257	0	BAS	70	MDMA	280	275	215	BAS
19	Procainamide	202	279	700	ACN	71	Nikethamide	280	260	280	ACN
20	Nizatidine	221	320	748	BAS	72	Phencyclidine	281	0	0	BAS
21	Ephedrine	224	0	0	BAS	73	Pemoline	281	0	0	BAS
22	Theobromine	224	272	560	BAS	74	Thiabendazole	283	302	1120	ACN
23	Atenolol	224	275	48	BAS	75	Ethylmorphine	283	282	48	BAS
24	MATRBAS2	226	0	0	BAS	76	Ethosuximide	284	0	0	ACN
25	Cimetidine	229	0	0	BAS	77	Lidocaine	285	0	0	BAS
26	Hydromorphone	231	282	50	BAS	78	Tetroxoprim	286	272	113	BAS
27	Amiloride	233	363	800	BAS	79	Pipamperone	286	248	315	BAS
28	Tryptamine	233	278	350	BAS	80	Barbital	287	0	0	ACN
29	Paracetamol	234	243	680	ACN	81	Atropine	287	259	17	BAS
30	Procaine	236	291	950	BAS	82	Scopolamine	288	0	0	BAS
31	Metronidazole	236	320	520	ACN	83	Metamizol	289	0	0	ACN
32	Dihydrocodeine	237	281	47	BAS	84	Triamterene	291	359	860	BAS
33	Clonidine	237	269	20	BAS	85	Strychnine	292	254	370	BAS
34	Nalorphine	237	284	50	BAS	86	Ketamine	294	270	25	BAS
35	Theophylline	239	270	540	BAS	87	8-Chlorotheophylline	295	267	604	BAS
36	Ranitidine	240	317	500	BAS	88	Ketobemidone	295	273	80	BAS
37	Sulpiride	240	291	70	BAS	89	Benzoylecgonine	295	274	30	BAS
38	Amphetamine	241	257	14	BAS	90	Timolol	297	297	279	BAS
39	Trancylpromine	241	261	20	BAS	91	Mepivacaine	300	0	0	BAS
40	Codeine	243	284	55	BAS	92	Piribedil	301	286	203	BAS
41	Aminophenazone	243	257	443	ACN	93	Harmane	301	299	0	BAS
42	MDE	244	285	200	BAS	94	Phenazone	303	256	450	ACN
43	Acetazolamide	249	264	475	ACN	95	Salicylamide	305	299	270	ACN
44	Naloxone	251	282	40	BAS	96	Nitrofurantoin	307	366	770	ACN
45	Tocainide	251	0	0	ACN	97	Guaifenesine	307	275	140	ACN
46	Saccharin	254	269	80	ACN	98	Quinine	308	246	950	BAS
47	Methamphetamine	255	255	15	BAS	99	Levorphanol	308	281	80	BAS
48	Proxibarbal	255	0	0	ACN	100	Metoclopramide	308	309	420	BAS
49	Mescaline	255	269	35	BAS	101	Primidone	308	0	0	ACN
50	Sulphadiazine	260	267	140	ACN	102	Acebutolol	311	233	655	BAS
51	Oxycodone	260	279	40	BAS	103	Phenacemide	312	0	0	ACN
52	MDA	261	285	200	BAS	104	Tramadol	314	270	70	BAS

Table 3 *(continued)*

No.	Substance	*I*	UV	A_1^1	Scale	No.	Substance	*I*	UV	A_1^1	Scale
105	Zopiclon	314	304	177	BAS	157	Brompheniramine	355	263	250	BAS
106	Acetanilide	316	239	815	ACN	158	Bupivacaine	355	0	0	BAS
107	Methyl phenidate	316	0	0	BAS	159	Phenacetin	356	246	650	ACN
108	Quinidine	316	245	950	BAS	160	Clopamide	356	240	346	ACN
109	Metoprolol	317	273	50	BAS	161	Phenobarbital	357	0	0	ACN
110	Pentoxifylline	320	275	314	BAS	162	Colchicine	357	351	420	BAS
111	Viloxazine	321	272	90	BAS	163	Chlordiazepoxide	357	306	316	ACN
112	Sulthiam	321	246	390	ACN	164	Pentazocine	357	278	70	BAS
113	Glafenine	322	345	467	ACN	165	LSD	358	315	0	BAS
114	Thebaine	324	285	250	BAS	166	Metapramine	358	270	181	BAS
115	Chloridazone	324	285	420	ACN	167	Trazodon	358	249	320	BAS
116	Buflomedil	324	276	460	BAS	168	Brallobarbital	359	0	0	ACN
117	Fenethylline	325	273	240	BAS	169	Chlorthalidone	359	275	58	ACN
118	Aspirin	326	274	68	ACN	170	Tripolidine	360	280	292	BAS
119	Disopyramide	327	261	195	BAS	171	Flumazenil	362	245	914	ACN
120	Melperone	327	248	415	BAS	172	Metipranolol	363	278	40	BAS
121	Diamorphine	327	276	46	BAS	173	Fenfluramine	364	264	22	BAS
122	Ajmaline	328	287	90	BAS	174	Secbutobarbital	365	0	0	ACN
123	Clozapine	330	294	650	BAS	175	Ketotifene	365	299	480	BAS
124	Salicylic acid	331	299	270	ACN	176	Butobarbital	365	0	0	ACN
125	Oxprenolol	332	0	0	BAS	177	Coumarin	368	315	607	ACN
126	Yohimbine	333	273	220	BAS	178	Droperidol	369	278	190	BAS
127	Pethidine	334	0	0	BAS	179	Carbutamide	369	267	149	ACN
128	Alimemazine sulphoxide	334	338	0	BAS	180	Clomethiazole	369	251	270	BAS
129	Dimethindene	334	260	613	BAS	181	Dextromethorphan	370	281	70	BAS
130	Flunitrazepam, 7-amino	336	236	0	ACN	182	Benzoctamine	370	271	57	BAS
131	Cocaine	336	275	30	BAS	183	Propranolol	370	290	220	BAS
132	Dimethoate	336	0	0	ACN	184	Benperidol	371	278	230	BAS
133	Tripelemaine	337	311	250	BAS	185	Betaxolol	371	275	76	BAS
134	Tilidine	337	0	0	BAS	186	Amoxapine	372	299	236	BAS
135	Hyoscine butyl bromide	338	0	0	BAS	187	Bupranolol	373	276	78	BAS
136	Norpethidine	339	0	0	BAS	188	Cyclobarbital	374	0	0	ACN
137	MAFB	339	235	0	ACN	189	Trichlormethiazide	377	267	466	ACN
138	Allobarbital	340	0	0	ACN	190	Phenelzine	377	0	0	BAS
139	Oxazolam	340	240	698	ACN	191	Fentanyl	377	0	0	BAS
140	Dexchlopheniramine	342	263	0	BAS	192	Cyclopentobarbital	377	0	0	ACN
141	Zolpidem	343	296	482	BAS	193	Bromazepam	378	317	65	ACN
142	MATRBAS3	344	258	0	BAS	194	Loprazolam	379	330	900	ACN
143	Morazone	344	261	200	BAS	195	MATRAC1	379	0	0	ACN
144	Diazoxide	345	264	436	ACN	196	Butalbital	380	0	0	ACN
145	Trimethoprim	345	270	220	ACN	197	Carbamazepine	380	284	490	BAS
146	Nomifensine	346	237	250	BAS	198	Benzocaine	380	284	930	BAS
147	Papaverine	346	249	1800	BAS	199	Tetracaine	381	314	600	BAS
148	Aprobarbital	347	0	0	ACN	200	Alprenolol	381	270	69	ACN
149	Dibenzepin	349	0	0	BAS	201	Mephenytoin	382	0	0	ACN
150	Labetalol	350	303	100	BAS	202	Chlormezanone	382	227	25	BAS
151	Trifluperidol 1	350	257	308	BAS	203	MATRBAS4	384	260	0	BAS
152	Etenzamide	350	293	220	ACN	204	Diphenhydramine	385	254	17	BAS
153	Buspirone	353	238	552	BAS	205	Paroxetin	385	294	0	BAS
154	Bromisoval	354	0	0	ACN	206	Midazolam	386	250	360	ACN
155	Carazolol	354	332	438	BAS	207	Buprenorphine	386	288	30	BAS
156	Noscapine	354	312	85	BAS	208	Moperon	387	245	564	BAS

(Continued on p. 106)

Table 3 *(continued)*

No.	Substance	*I*	UV	A_1^1	Scale	No.	Substance	*I*	UV	A_1^1	Scale
209	Opipramol	387	254	870	BAS	262	Imipramine	434	251	300	BAS
210	Dipyridamole	387	284	650	BAS	263	Trifluperidol 2	436	246	0	BAS
211	Demoxepam	388	308	600	ACN	264	Secobarbital	437	0	0	ACN
212	Digoxin	389	0	0	BAS	265	Alimemazine	437	252	0	BAS
213	Mianserine	390	278	75	BAS	266	Perphenazine	438	255	800	BAS
214	Projmaline	390	246	198	BAS	267	Dextropropoxyphene	438	255	12	BAS
215	Dilthiazem	392	238	661	BAS	268	Pecazine	440	252	900	BAS
216	Flurazepam	392	309	50	ACN	269	Dextromoramide	440	0	0	BAS
217	Medazepam	395	254	860	ACN	270	Maprotiline	440	0	0	BAS
218	Idobutal	395	0	0	ACN	271	Oxazepam	441	228	1200	ACN
219	Piritramid	395	0	0	BAS	272	Methadon	443	0	0	BAS
220	Clotiapine	397	299	174	BAS	273	Alprazolam	443	276	938	ACN
221	Fluanisone	398	244	550	BAS	274	Isosorbide dinitrate	445	0	0	BAS
222	Carpipramine	399	251	184	BAS	275	Amitriptyline	446	239	500	BAS
223	Acepromazine	399	278	570	BAS	276	Levomepromazine	446	305	140	BAS
224	Loxapine	399	296	184	BAS	277	Indole	448	270	0	BAS
225	Carbromal	400	0	0	ACN	278	Fluoxetine	450	263	130	BAS
226	Doxepin	401	294	230	BAS	279	Atrazine	451	264	180	ACN
227	Demeton-S-methyl	402	0	0	ACN	280	Clonazepam	451	309	350	ACN
228	Hexobarbital	404	0	0	ACN	281	Trimipramine	451	249	300	BAS
229	Heptabarbital	404	0	0	ACN	282	Triazolam	452	220	1100	ACN
230	MATRAC3	404	323	0	ACN	283	Carbaryl	452	280	351	ACN
231	Periciazine	405	269	780	BAS	284	Glisoxepide	453	230	70	ACN
232	Pentobarbital	405	0	0	ACN	285	Verapamil	454	278	130	BAS
233	Propafenone	408	304	107	BAS	286	Monolinuron	455	246	1130	ACN
234	Haloperidol	409	245	340	BAS	287	Methaqualone	455	266	350	BAS
235	Flecainide	410	298	294	BAS	288	Clopenthixol	456	270	370	BAS
236	Vinylbarbital	410	0	0	ACN	289	DCFB	458	236	0	ACN
237	Promethazine	411	249	1050	BAS	290	Flunitrazepam	459	309	330	ACN
238	Fluvoxamine	412	252	340	BAS	291	Desalkylflurazepam	459	230	0	ACN
239	Furosemide	414	275	600	ACN	292	Naftidrofuryl	460	282	141	BAS
240	Amobarbital	415	0	0	ACN	293	ABP, 1st peak	461	327	0	ACN
241	Phenytoin	415	0	0	ACN	294	Benztropine	461	0	0	BAS
242	Orphenadrine	416	0	0	BAS	295	Prochlorperazine	462	257	880	BAS
243	Normethadone	417	0	0	BAS	296	Clorazepate	464	311	55	ACN
244	Promazine	418	252	1060	BAS	297	Bromoxynil	464	255	380	ACN
245	Flunitrazepam, 3-hydroxy	418	311	0	ACN	298	Glipizide	464	275	230	ACN
246	Perazine	418	252	900	BAS	299	Nordiazepam	464	230	1200	ACN
247	Nortriptyline	418	239	989	BAS	300	Etomidate	466	237	450	ACN
248	Methylphenobarbital	420	0	0	ACN	301	Chlorpromazine	466	254	1030	BAS
249	Desipramine	421	251	308	BAS	302	Temazepam	466	228	1100	ACN
250	Propyphenazone	422	272	400	ACN	303	Diuron	468	250	900	ACN
251	Lorazepam	422	230	1100	ACN	304	Tolbutamide	470	228	581	ACN
252	Acecarbromal	422	0	0	ACN	305	Clomipramine	471	254	250	BAS
253	Protriptyline	424	290	530	BAS	306	Reserpine	473	294	200	BAS
254	Estrazolam	424	0	0	ACN	307	Lormetazepam	474	229	1030	ACN
255	Flunitrazepam, nor	424	312	0	ACN	308	Tiaprofenic acid	475	306	570	ACN
256	Piroxicam	425	363	420	ACN	309	Chlorprothixene	476	267	420	BAS
257	Hydroxyzine	428	230	420	BAS	310	Naproxene	476	262	200	ACN
258	Dothiepin	428	260	260	BAS	311	Paraoxon	479	270	249	ACN
259	Cinchocaine	430	327	190	BAS	312	Fluphenazine	480	307	85	BAS
260	Nitrazepam	430	311	364	ACN	313	Thiopental	481	285	790	ACN
261	Gluthetimide	430	0	0	ACN	314	Bisacodyl	483	264	270	ACN

Table 3 *(continued)*

No.	Substance	I	UV	A_1^1	Scale	No.	Substance	I	UV	A_1^1	Scale
315	Clobazam	484	293	75	ACN	350	Tebuconazole	607	0	0	ACN
316	Flupenthixol	487	267	294	BAS	351	Phenprocoumon	609	310	450	ACN
317	Trifluoperazine	491	255	750	BAS	352	Nitrendipine	610	236	1340	ACN
318	Terbutryn	494	260	370	ACN	353	Indomethacin	610	317	180	ACN
319	ABP, 2nd peak	495	237	0	ACN	354	Metolachlor	618	0	0	ACN
320	Butaperazine	496	278	540	BAS	355	Parathion-methyl	621	275	238	ACN
321	Oxyphenbutazone	503	237	1400	ACN	356	Glibenclamide	623	302	63	ACN
322	Nifedipine	503	275	0	ACN	357	Propoxur 2	624	264	84	ACN
323	Methohexital	503	0	0	ACN	358	Diclofenac	630	275	320	ACN
324	Thioridazine	504	261	1000	BAS	359	MNFB 2nd peak	630	231	0	ACN
325	Triflupromazine	505	257	992	BAS	360	Nimodipine	641	237	0	ACN
326	Ethacrynic acid	516	276	100	ACN	361	Prazepam	648	227	1800	ACN
327	MATRBAS1	520	279	0	BAS	362	Ibuprofen	650	0	0	ACN
328	Fenbufen	522	285	835	ACN	363	Phenylbutazone	651	237	450	ACN
329	MNFB, 1st peak	525	230	0	ACN	364	Demeton-S-methylsulphone	660	0	0	ACN
330	Diazepam	529	229	1100	ACN	365	Edifenphos	660	0	0	ACN
331	Nitroglycerine	531	0	0	ACN	366	ACB	673	236	310	ACN
332	Tetrazepam	538	228	1100	ACN	367	Diazinon	687	247	35	ACN
333	Gliclazide	538	261	20	ACN	368	ADB	704	233	0	ACN
334	Propoxur 1	540	264	84	ACN	369	Penfluridol	716	270	50	BAS
335	ANB	549	365	0	ACN	370	MATRAC2	720	279	0	ACN
336	Clotiazepam	551	243	580	ACN	371	Gliquidone	723	311	50	ACN
337	Warfarin	555	305	450	ACN	372	Disulfiram	730	279	680	BAS
338	ANFB	559	357	0	ACN	373	Chlorpyrifos	745	288	155	ACN
339	Linuron	559	249	900	ACN	374	MDB	750	265	0	ACN
340	Probenecid	560	249	319	ACN	375	Disulfoton	752	0	0	ACN
341	Camazepam	566	314	80	ACN	376	Trifluralin	761	274	248	ACN
342	Terfenadine	567	0	0	BAS	377	Amiodarone	762	240	1200	BAS
343	Flunarizine	571	252	599	BAS	378	Bromophos-ethyl	800	0	0	ACN
344	ANCB	580	356	0	ACN	379	Trichlorfon	800	0	0	ACN
345	Ketazolam	583	242	500	ACN	380	MACB	826	236	0	ACN
346	Niflumic acid	586	288	800	ACN	381	CCMK	900	271	0	ACN
347	Fenoprofen	587	0	0	ACN	382	Δ^9-THC	999	279	40	ACN
348	Spironolactone	594	287	570	ACN	383	CCB	999	237	0	ACN
349	Meclozine	601	228	390	BAS						

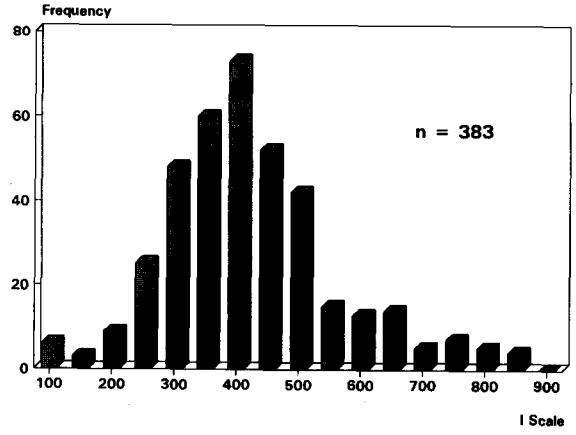

Fig. 3. Distribution of retention index values of all substances in the HPLC database.

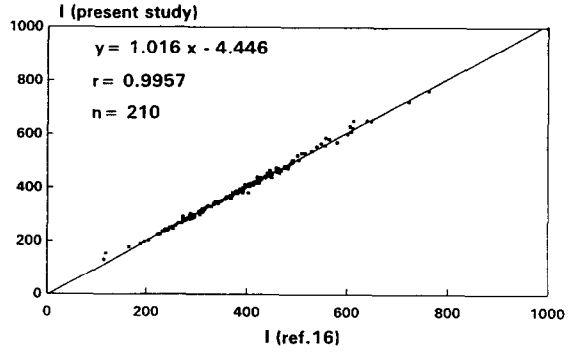

Fig. 4. Correlation of retention index values of 210 compounds obtained in two laboratories under standardized conditions.

ditions and using the same make of column, but in another laboratory and by means of different instrumentation; 210 of these substances were also examined in this study. This gave an opportunity to compare the I values, which were very consistent. The differences between the I values obtained for the same substances in two laboratories were, as a rule, less than 10 units. Similar reproducibility was observed for duplicate measurements in a single laboratory. The correlation graph of these data is shown in Fig. 3.

The UV spectra for 190 selected drugs are shown in Figs. 4 and 5. The reason for present-

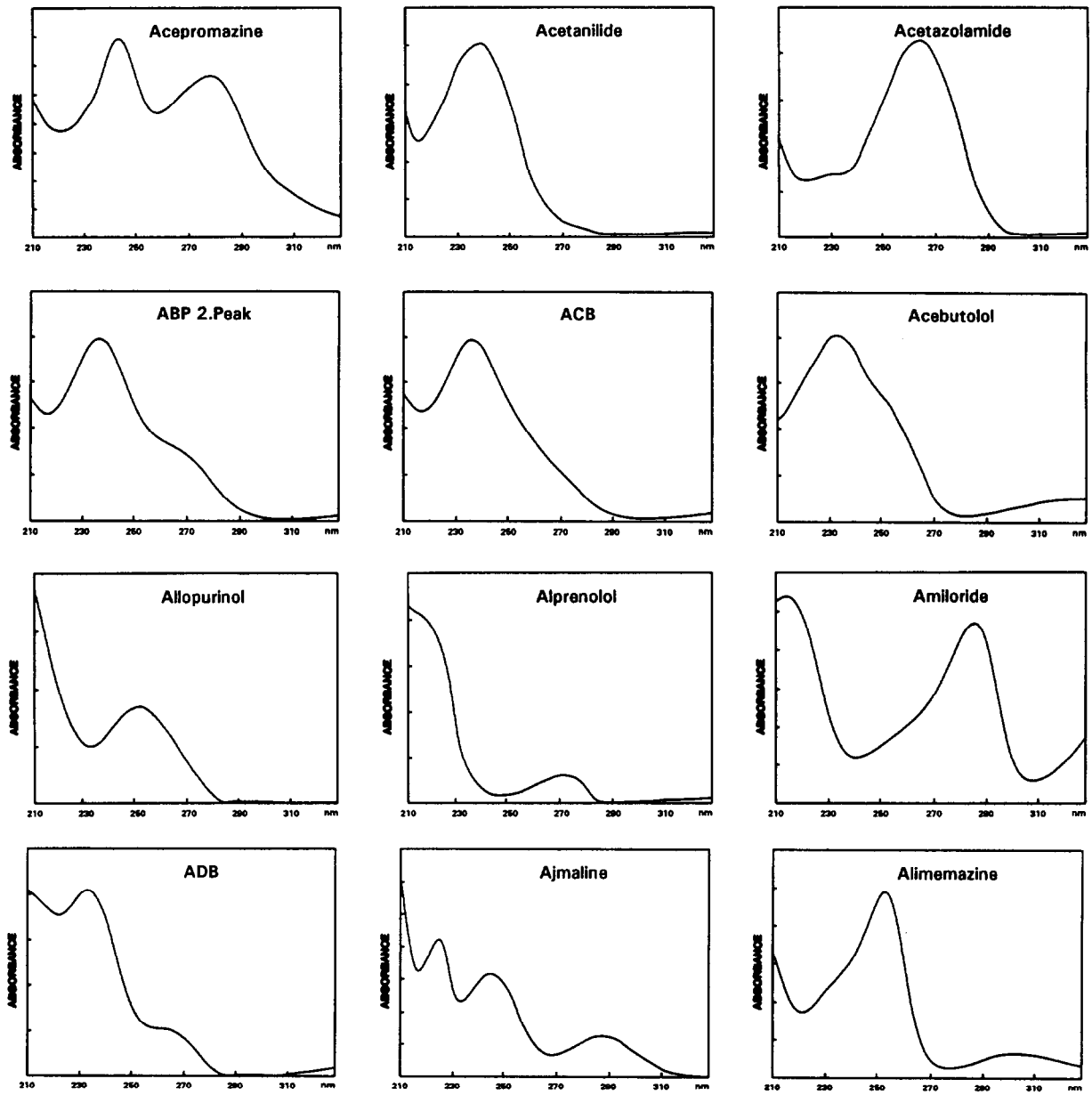

Fig. 5. UV spectra of selected drugs recorded during chromatographic runs and stored in the HPLC database.

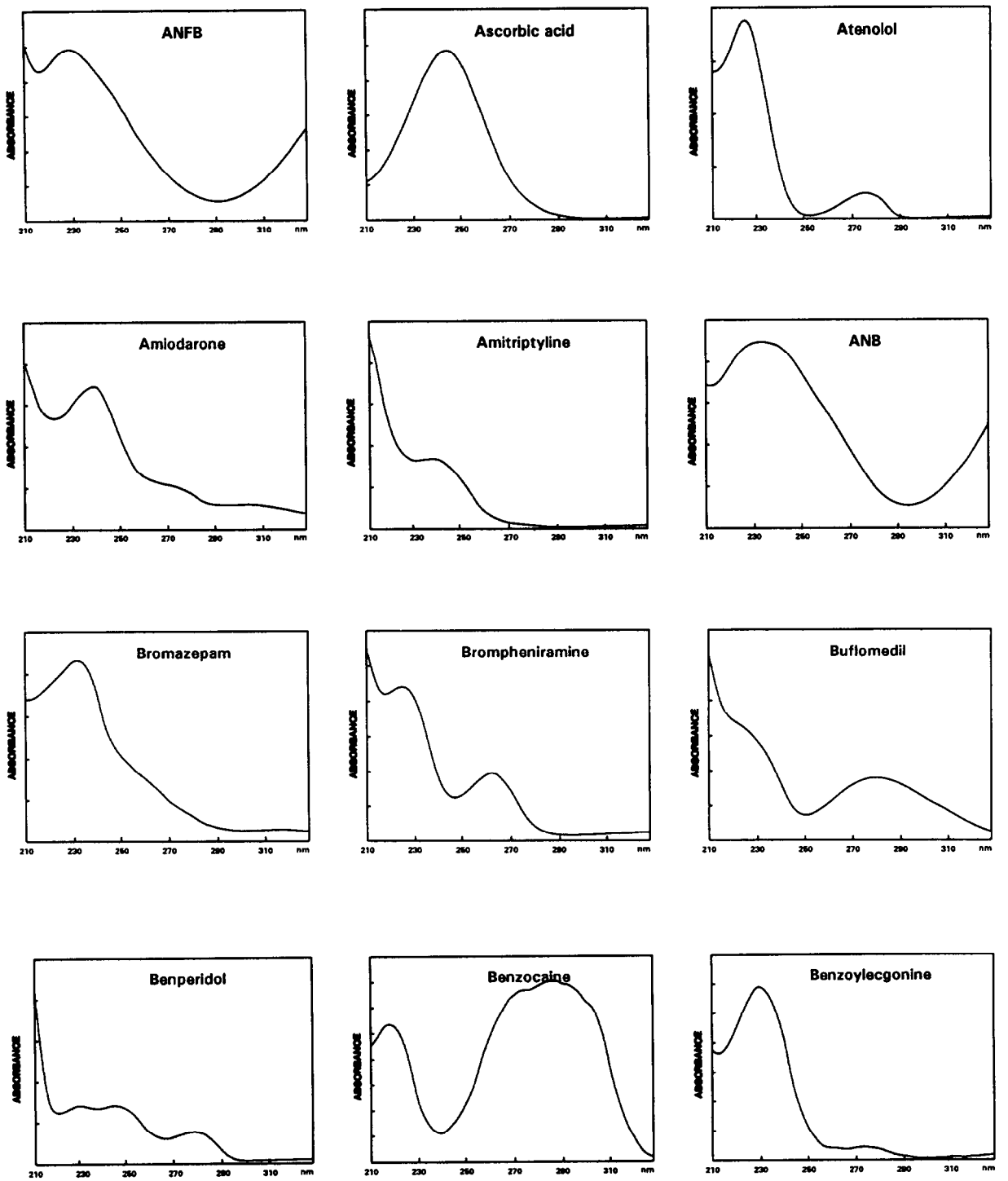

Fig. 5. *(Continued on p. 110)*

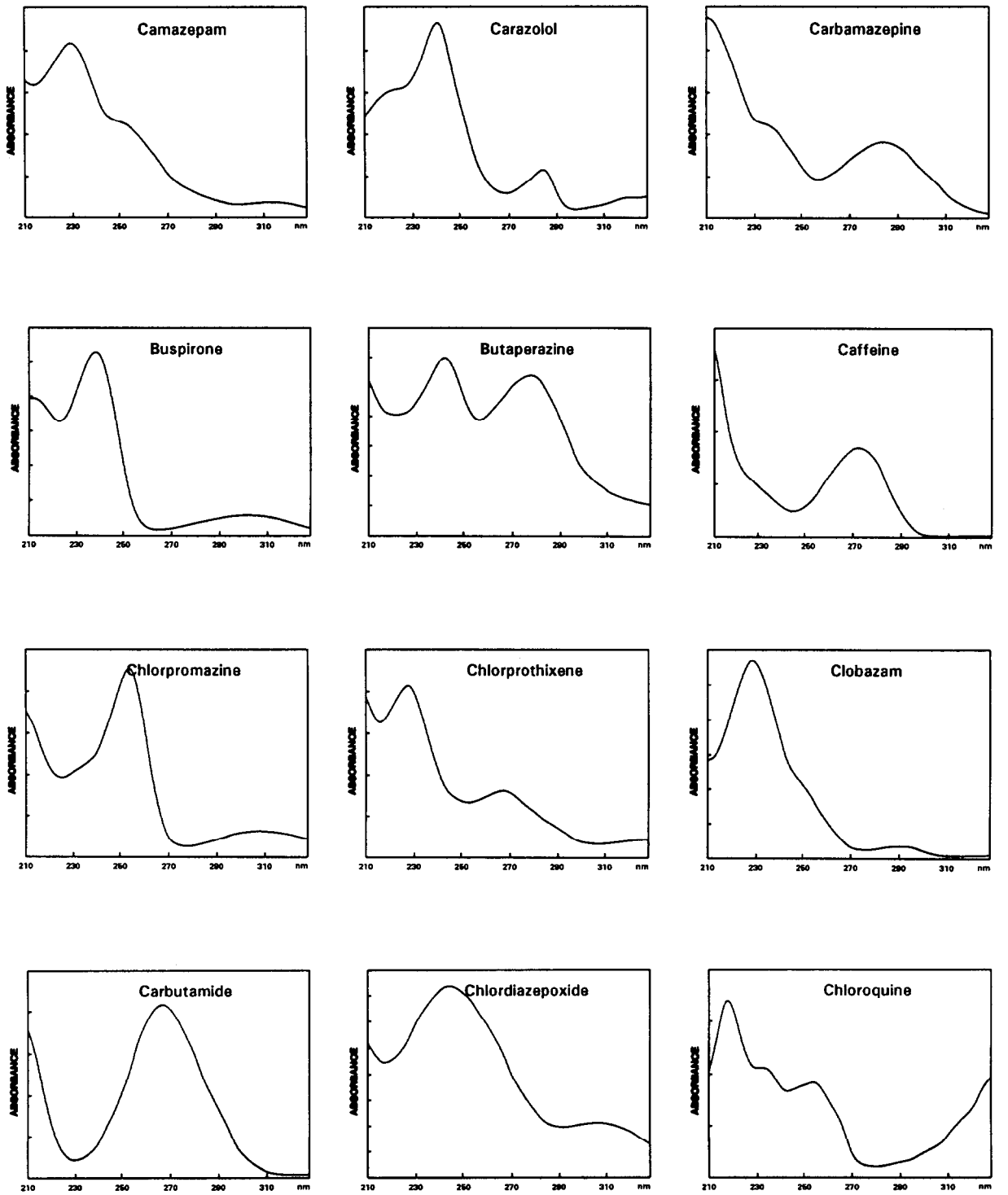
Camazepam
Carazolol
Carbamazepine
Buspirone
Butaperazine
Caffeine
Chlorpromazine
Chlorprothixene
Clobazam
Carbutamide
Chlordiazepoxide
Chloroquine
ABSORBANCE
210 230 250 270 290 310 nm

Fig. 5.

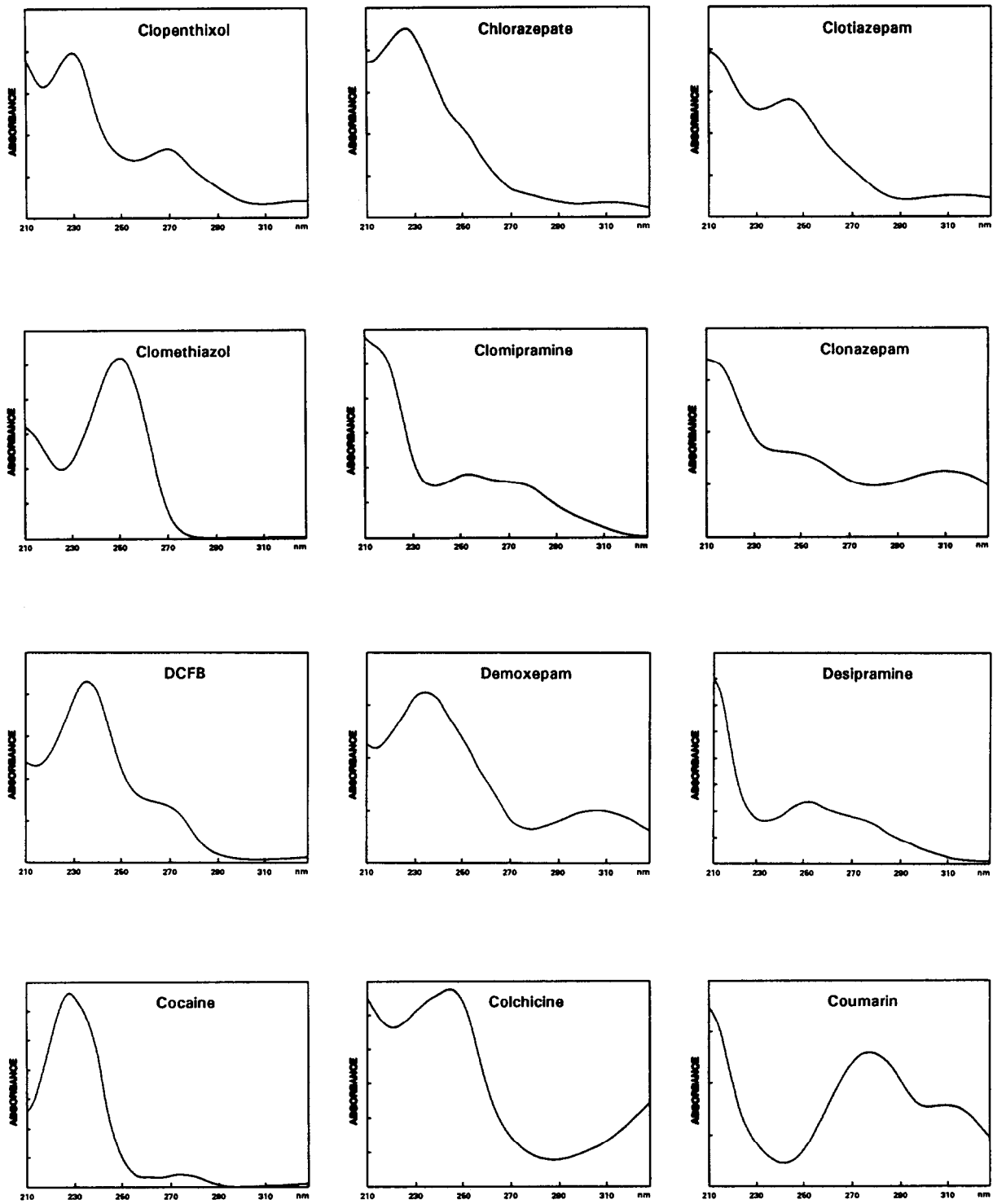

Fig. 5. *(Continued on p. 112)*

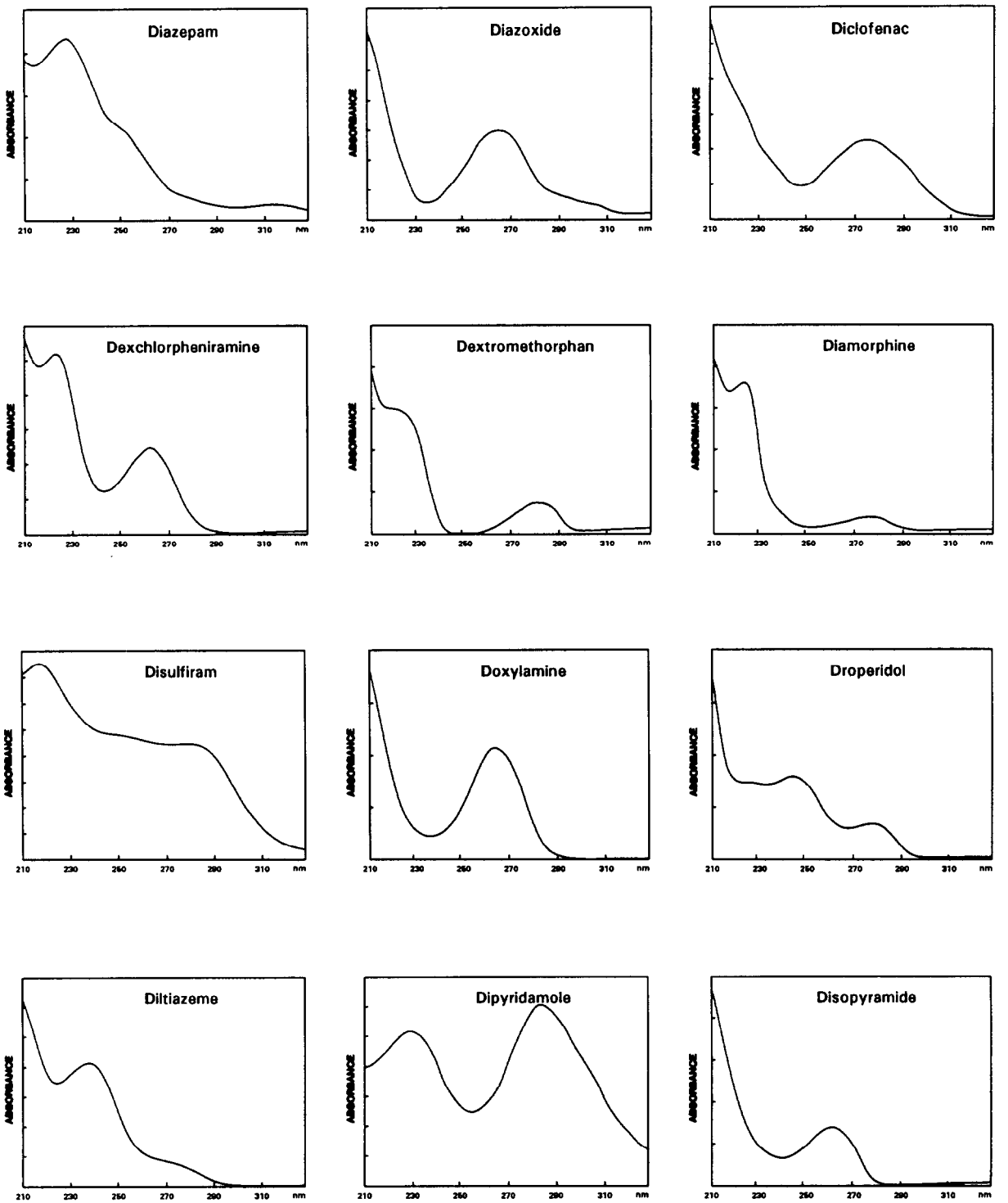
Diazepam
Diazoxide
Diclofenac
Dexchlorpheniramine
Dextromethorphan
Diamorphine
Disulfiram
Doxylamine
Droperidol
Diltiazeme
Dipyridamole
Disopyramide
ABSORBANCE
210 230 250 270 290 310 nm

Fig. 5.

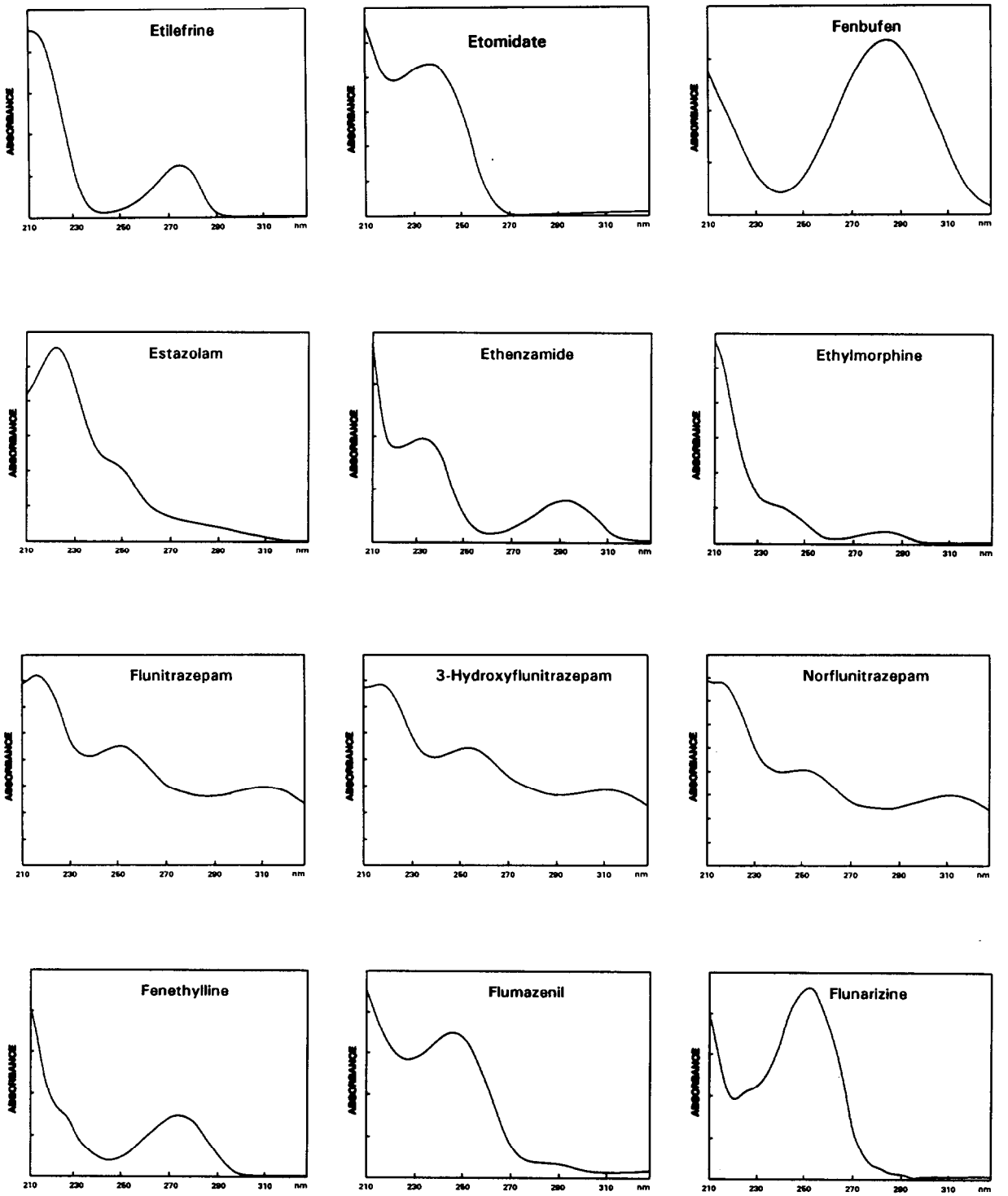

Fig. 5. *(Continued on p. 114)*

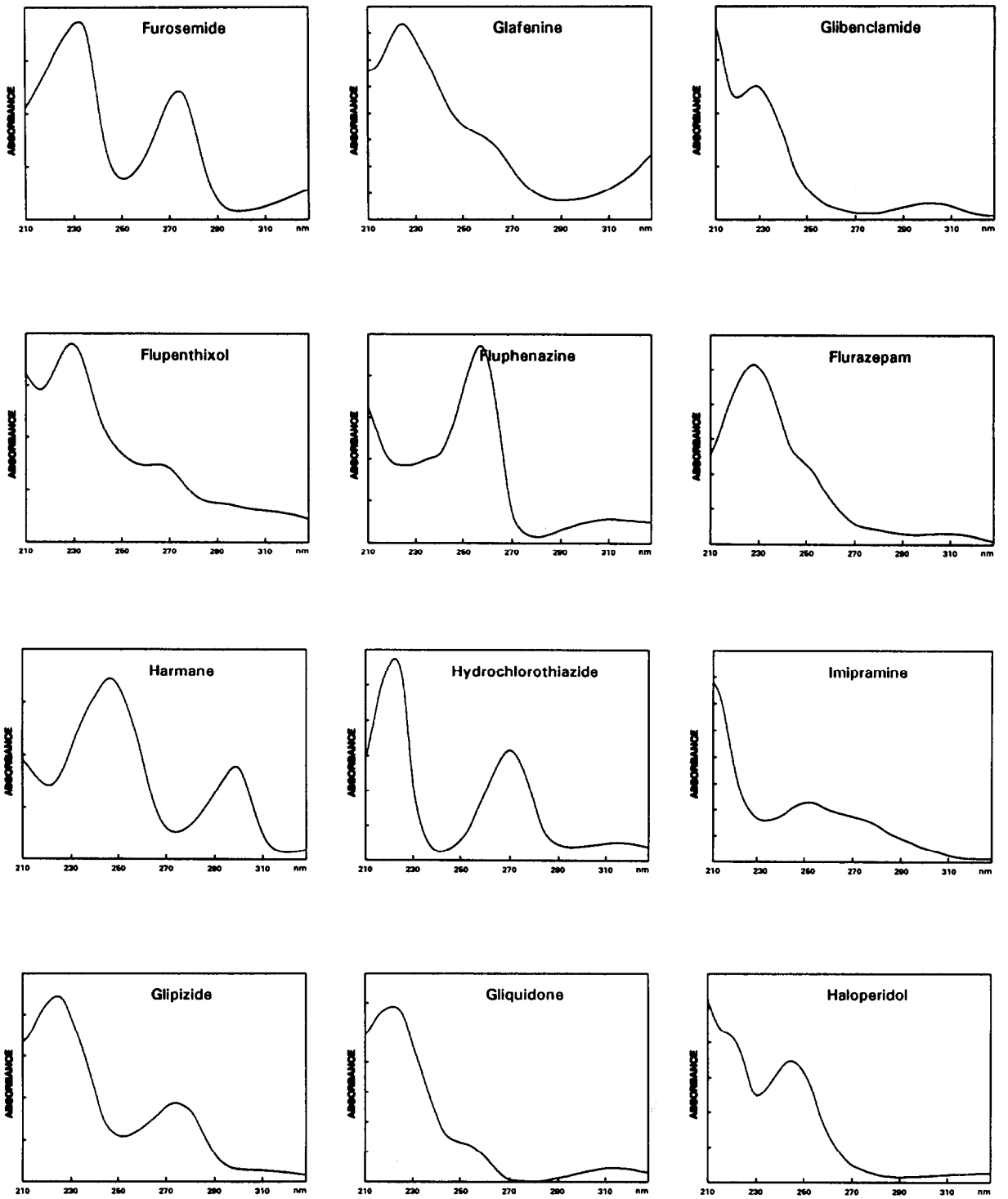
Furosemide
Glafenine
Glibenclamide
Flupenthixol
Fluphenazine
Flurazepam
Harmane
Hydrochlorothiazide
Imipramine
Glipizide
Gliquidone
Haloperidol
ABSORBANCE
210 230 260 270 290 310 nm

Fig. 5.

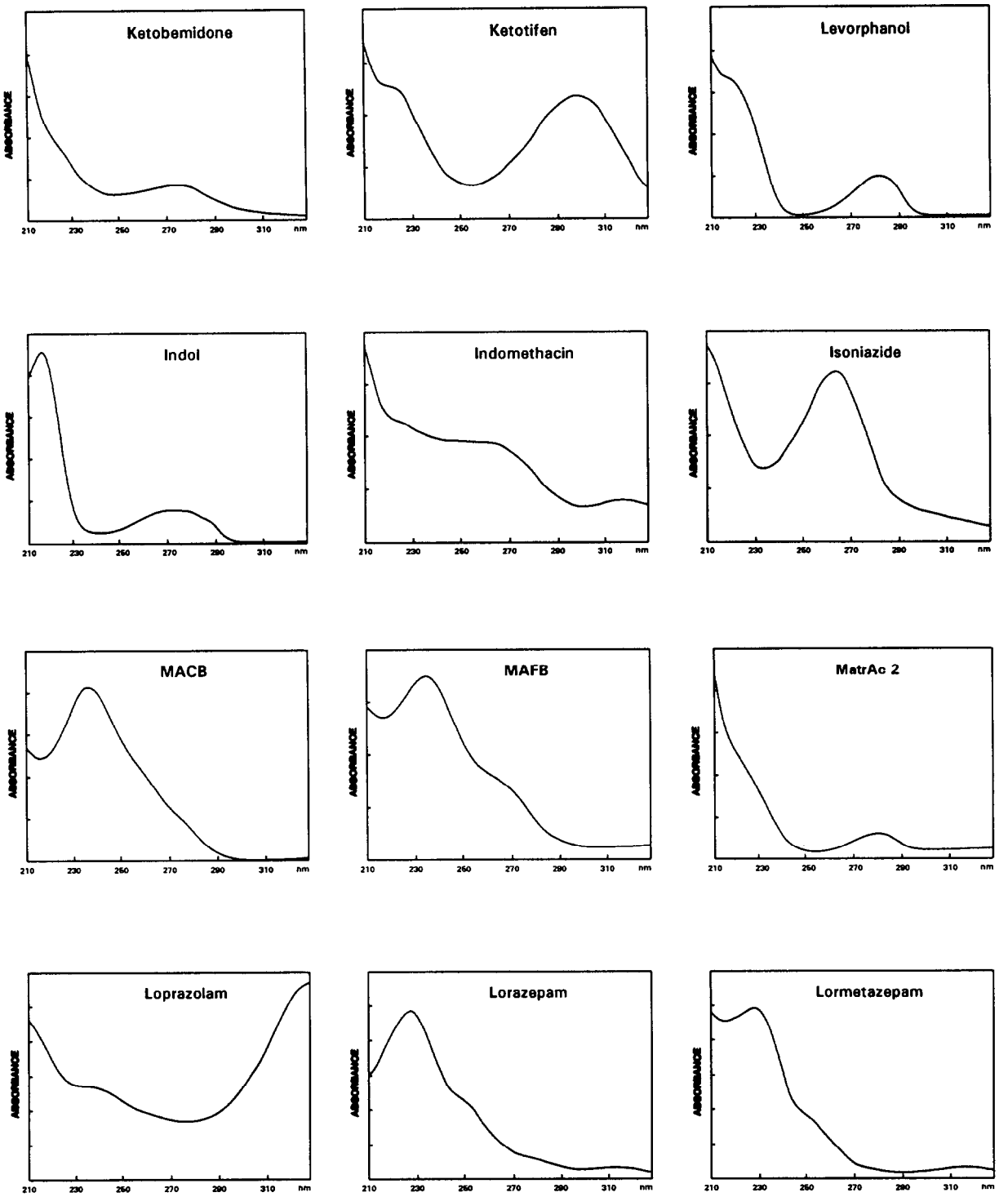

Fig. 5. *(Continued on p. 116)*

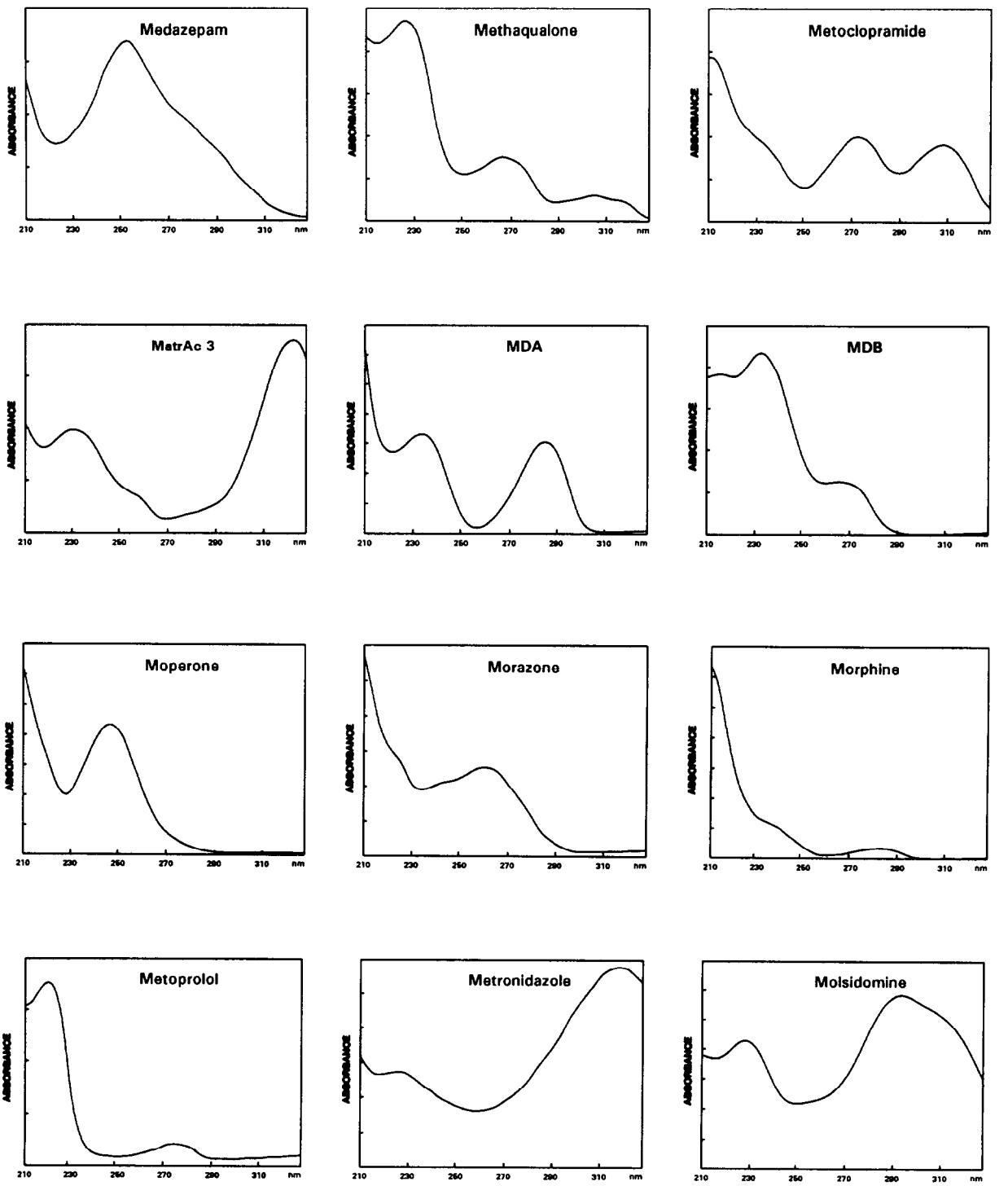
Medazepam
Methaqualone
Metoclopramide
MatrAc 3
MDA
MDB
Moperone
Morazone
Morphine
Metoprolol
Metronidazole
Molsidomine
ABSORBANCE
210 230 250 270 290 310 nm

Fig. 5.

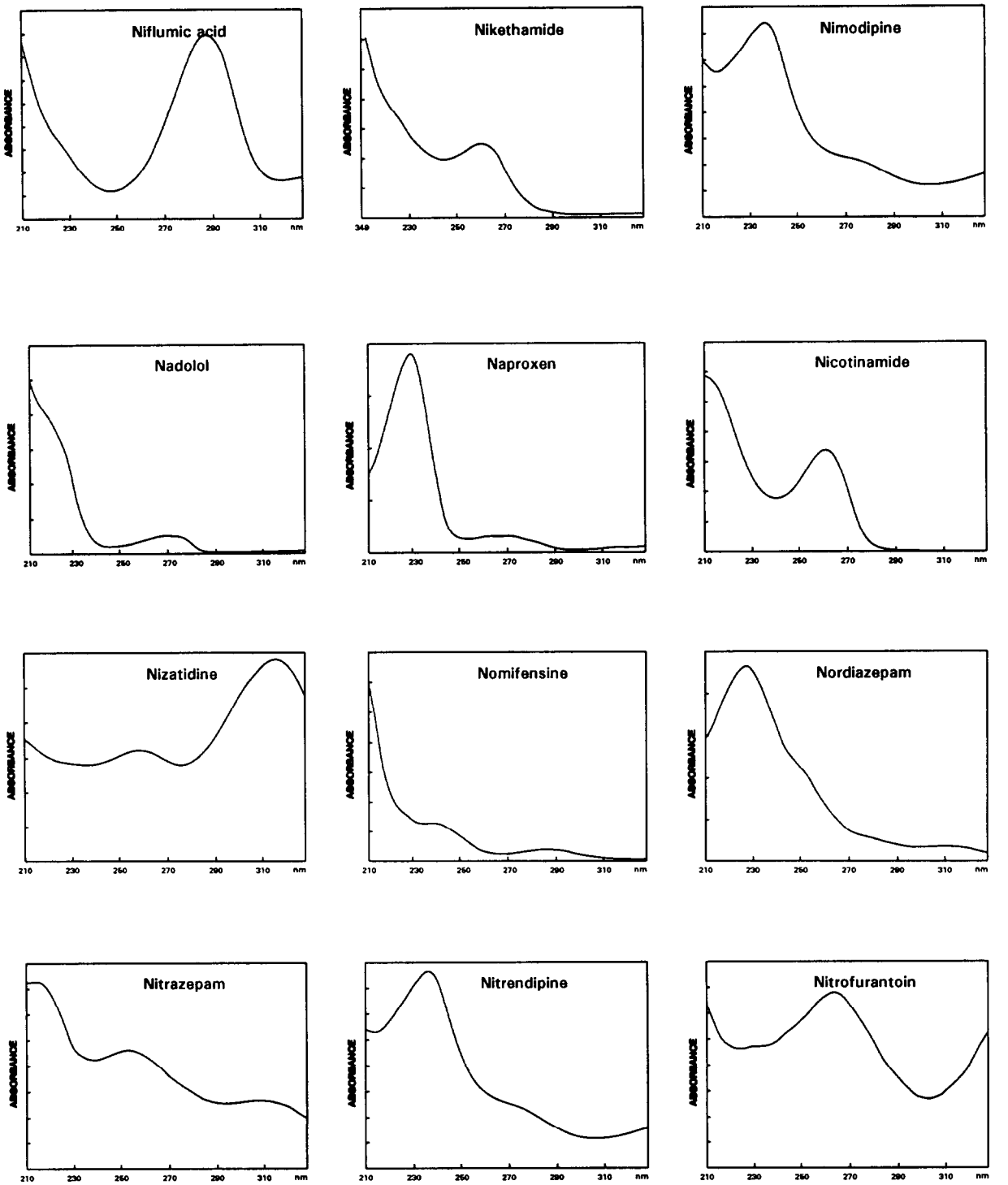

Fig. 5. *(Continued on p. 118)*

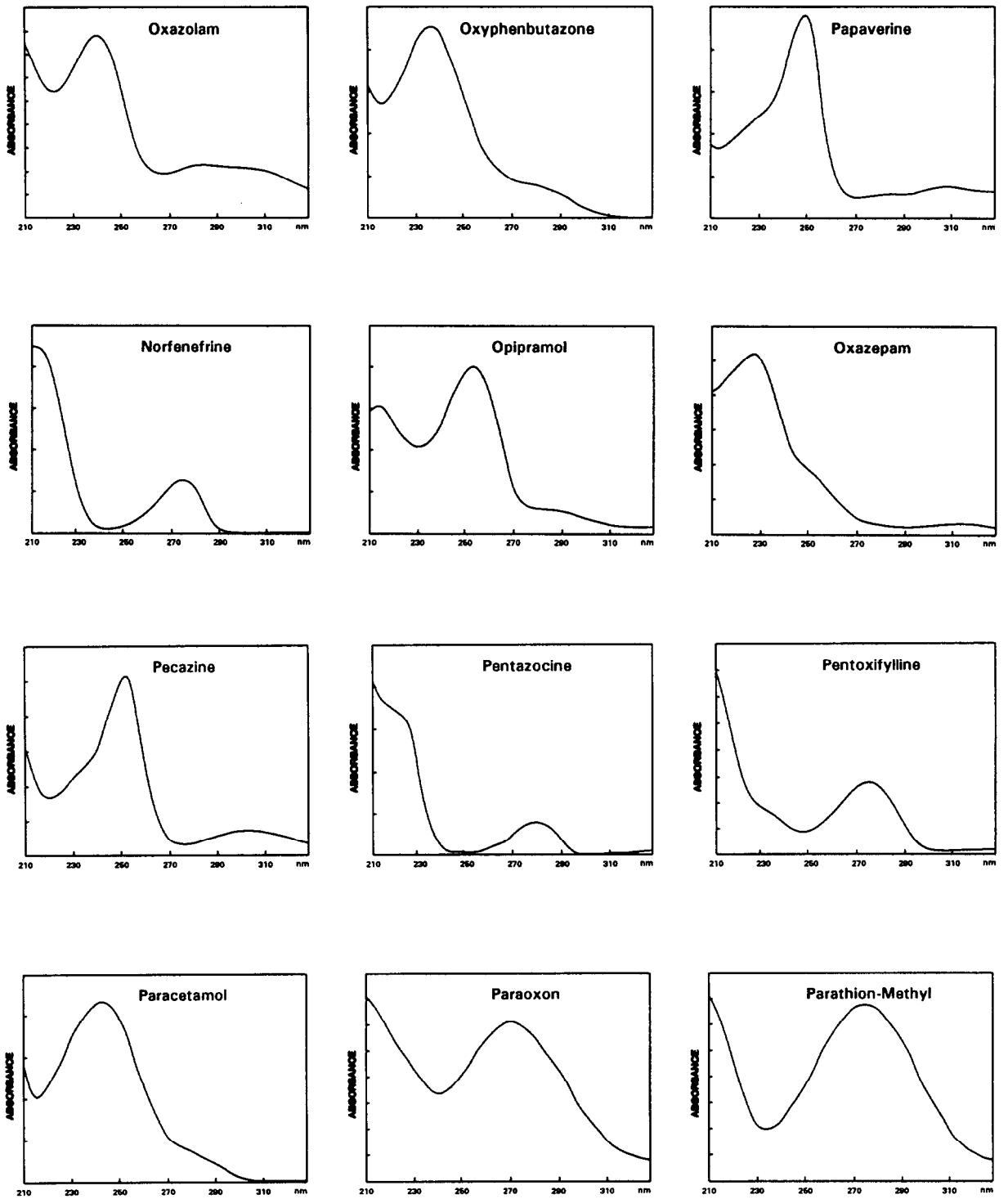
Oxazolam
Oxyphenbutazone
Papaverine
Norfenefrine
Opipramol
Oxazepam
Pecazine
Pentazocine
Pentoxifylline
Paracetamol
Paraoxon
Parathion-Methyl
ABSORBANCE
210 230 250 270 290 310 nm

Fig. 5.

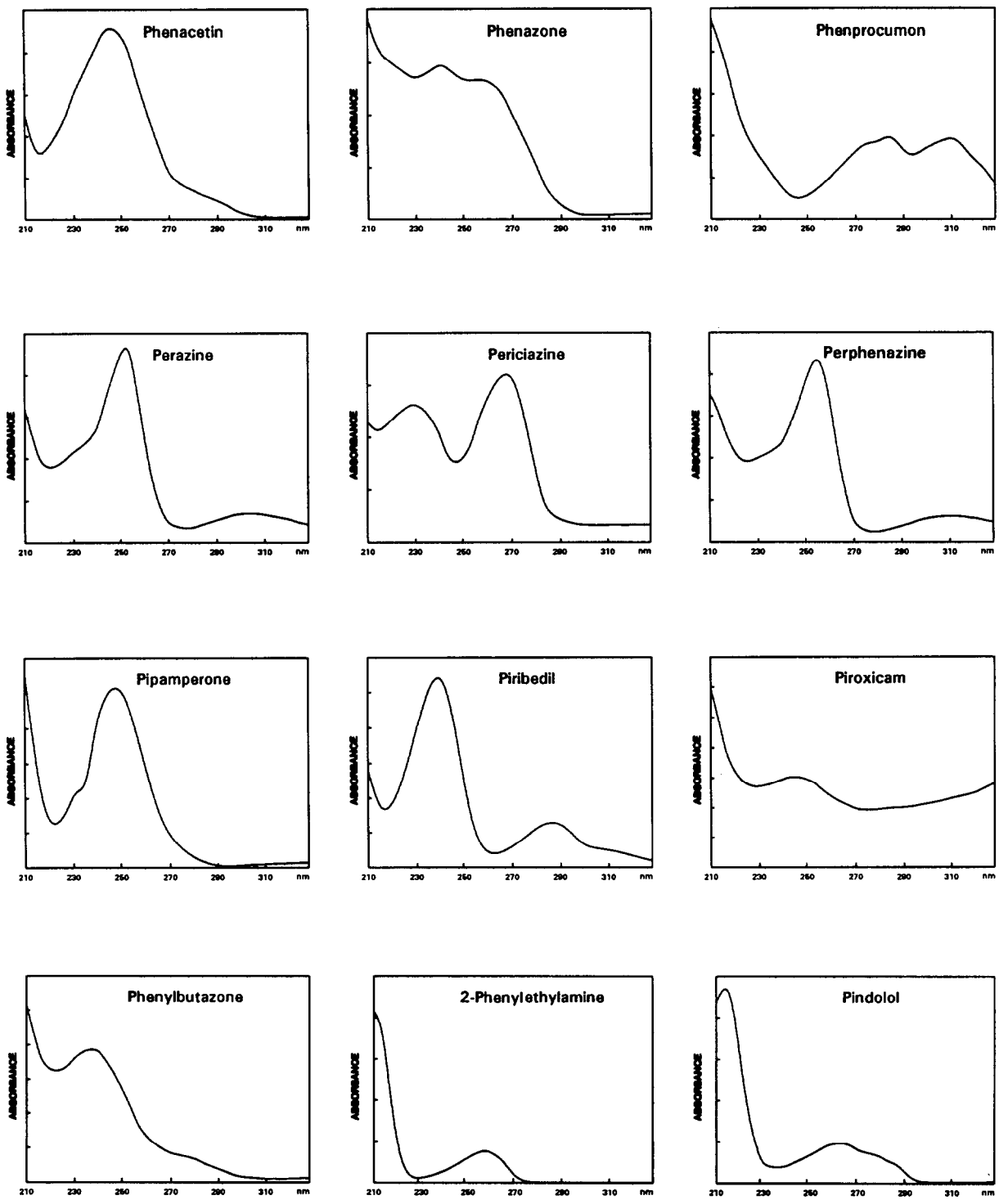

Fig. 5. *(Continued on p. 120)*

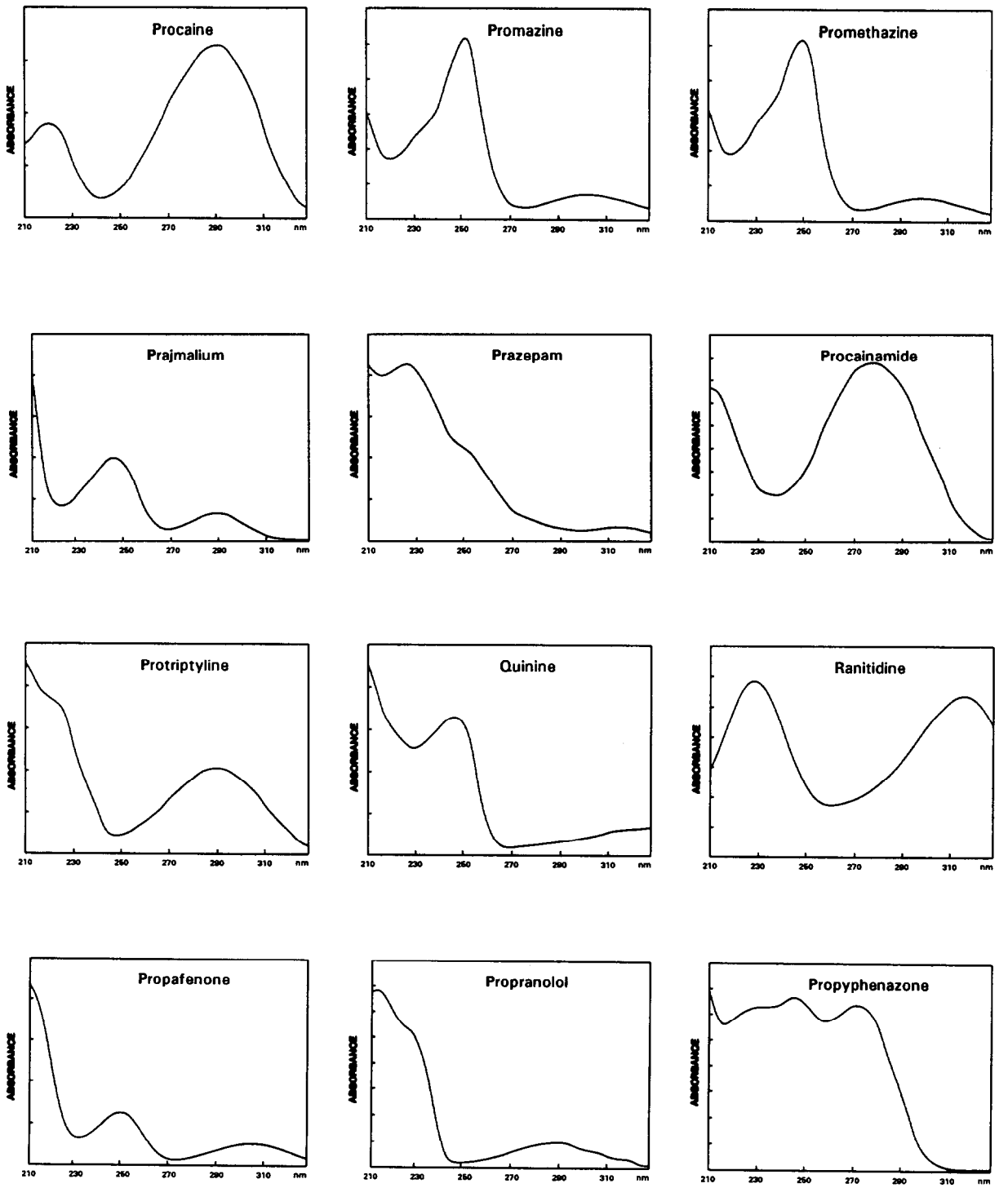
Procaine
Promazine
Promethazine
Prajmalium
Prazepam
Procainamide
Protriptyline
Quinine
Ranitidine
Propafenone
Propranolol
Propyphenazone
ABSORBANCE
210 230 250 270 290 310 nm

Fig. 5.

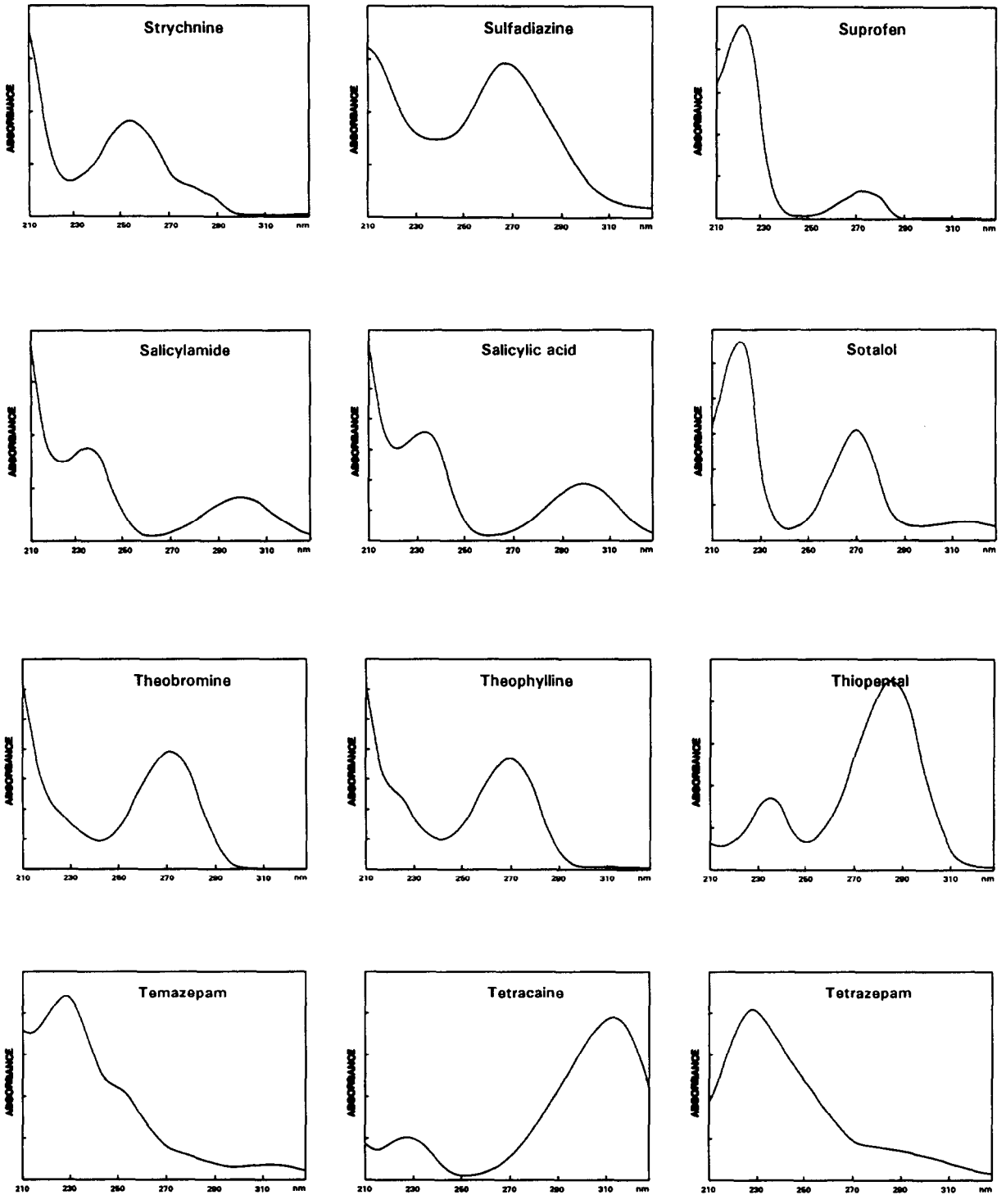

Fig. 5. *(Continued on p. 122)*

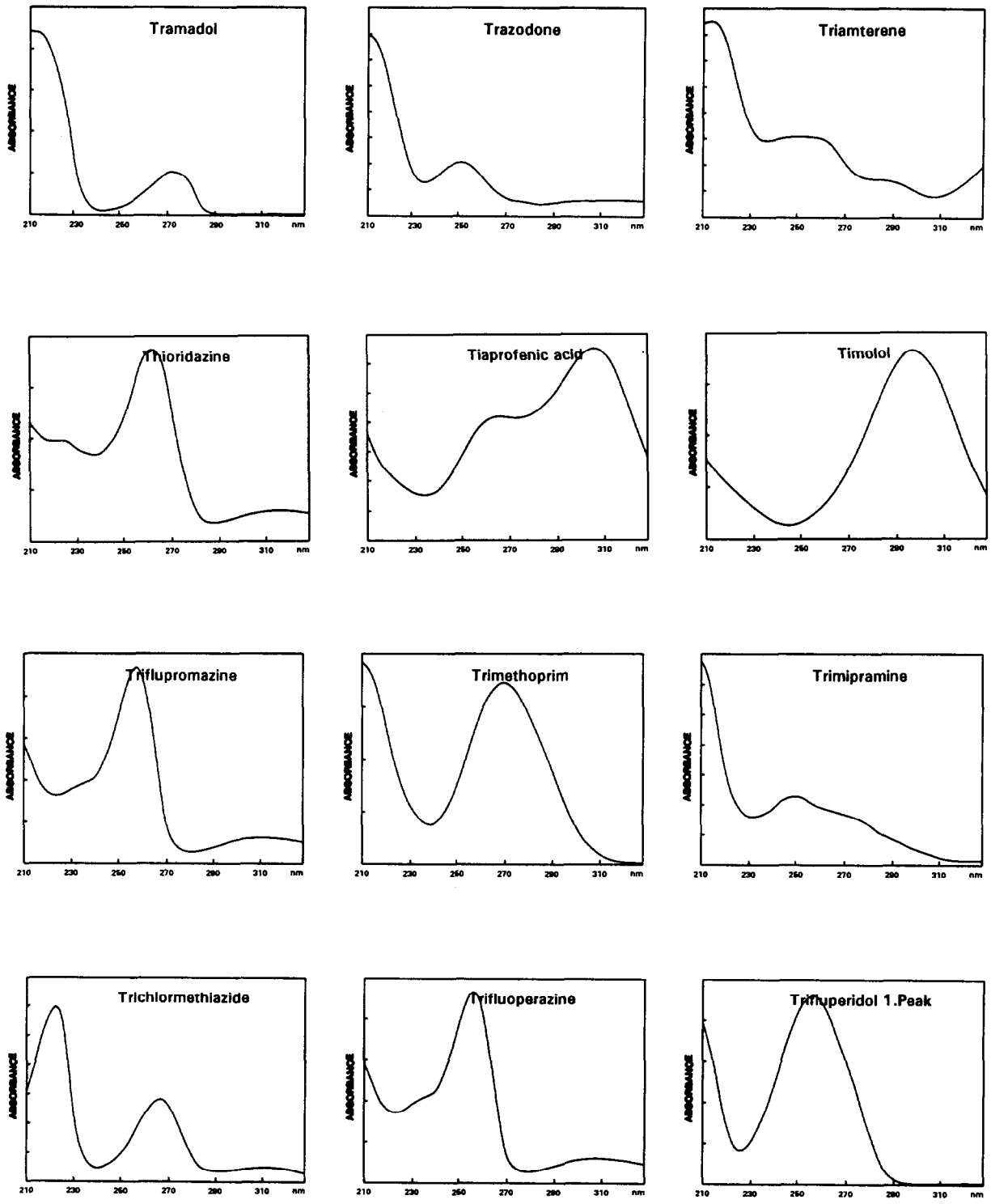
Tramadol
Trazodone
Triamterene
Thioridazine
Tiaprofenic acid
Timolol
Trifluproma zine
Trimethoprim
Trimipramine
Trichlormethiazide
Trifluoperazine
Trifluperidol 1.Peak
ABSORBANCE
210 230 250 270 290 310 nm

Fig. 5.

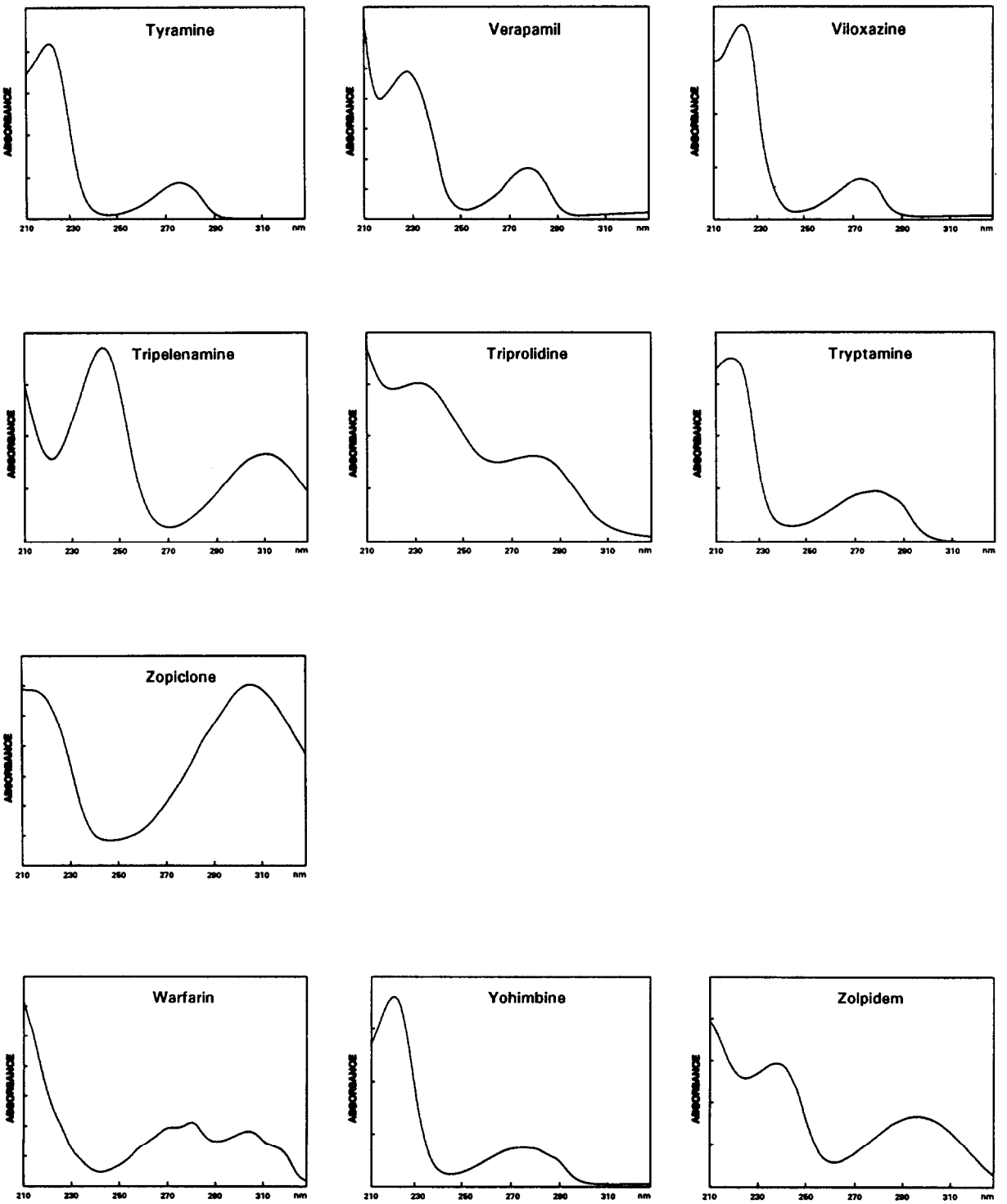
Tyramine
Verapamil
Viloxazine
Tripelenamine
Triprolidine
Tryptamine
Zopiclone
Warfarin
Yohimbine
Zolpidem
ABSORBANCE
210 230 250 270 290 310 nm

Fig. 5.

ing the spectra is that not all spectra which were measured at pH 3.0 are identical with the spectra measured in acidic solution, *e.g.*, in 0.1 *M* HCl. Comparison of several spectra with those published in a standard source [27] showed that some of them correspond to the spectra measured in alkaline medium. The "alkaline" spectra were observed for benzodiazepine derivatives (clonazepam, clorazepate, diazepam, flurazepam, nitrazepam, nordiazepam, oxazepam, prazepam, temazepam and tetrazepam), for some local anaesthetics (benzocaine, cinchocaine, procaine), antidepressant drugs (amoxapine, loxapine, nomifensine) and others (clomethiazole, clopamide, gliclazide, metronidazole, morazone, niflumic acid, propyphenazone, salicylic acid). Additionally, numerous substances produced spectra that were intermediate between acidic and alkaline types. These differences were caused by two factors: the different pH values of the mobile phase and of 0.1 *M* HCl and the influence of triethylammonium ion. Koves and Wells [14] also observed "alkaline" spectra for diazepam and nordiazepam using a mobile phase of pH 3.4 and containing triethylamine.

4. Discussion

The various factors relevant for the successful application of HPLC identification system are discussed in turn.

4.1. Intra- and interlaboratory reproducibility of results

The reproducibility of retention parameters, stored in the database, determines the identification potential of an identification system [28]. Therefore, various aspects of the reproducibility of the presented HPLC identification system were subjected to separate studies. Comparison of *I* values of 39 acidic and 109 basic drugs, obtained in two laboratories using two different ODS columns and different instrumental sets, demonstrated good reproducibility of the results, with mean standard deviations of 6.9 and 8.4 *I* units for acidic and basic drugs, respectively [29]. The interlaboratory study with application of seven different reversed-phase columns (octyl- and octadecylsilica) revealed an interlaboratory mean standard deviation of 10.3 *I* units [24]. This level of reproducibility was comparable with values observed for the same column packing in different laboratories, as shown by the comparison of the present results with those observed in the previous study [16].

The identification potential of the presented HPLC system was assessed by means of the "discriminating power" [30] and "mean list length" [31] approaches. It was demonstrated that the combination of retention indices and UV data shows a similar identification potential to the combination of capillary GC and off-line UV spectrophotometry [32].

4.2. Applicability of HPLC library to biological samples

The present HPLC database was established with pure drugs. It was therefore important to know whether the co-extracted biological matrix might influence the chromatographic behaviour of drugs in the extract, and to what extent the detectability of toxicologically relevant substances might be affected by endogenous matrix substances. This problem was investigated by Neidl-Fischer [33], who demonstrated that the *I* values of barbiturates and of doxepin, extracted from biological material in authentic cases of poisoning, were virtually identical with the listed values. Also, our own study showed that the chromatographic mobility of selected acidic and basic drugs is not significantly affected by endogenous material, extracted from serum, blood or liver [34]. It is therefore possible to use the *I* values or UV spectra taken from pure compounds for the identification of extracted substances. Biological extracts may, however, contain various endogenous substances of high UV absorbance and similar chromatographic mobility to various drugs. These substances may over-

lap the elution regions of toxicologically relevant substances and affect the identification potential. Therefore, the chromatographic purity of extracts is extremely important for identification and several workers have developed isolation methods that are particularly suitable for HPLC screening with diode-array detection [12–14]. The database contains some identified endogenous compounds, such as indole, tryptamine and phenylethylamine, and unidentified matrix substances of acidic or basic character.

4.3. Selectivity and sensitivity of detection

It is obvious that the identification potential of the HPLC system depends on the chromatographic quality of the column used. In previous studies we demonstrated that fully end-capped reversed-phase columns (octyl- or octadecylsilica) are very suitable for this kind of analysis [24,34]. Nevertheless, the possible future development of capillary HPLC, together with suitable detection systems, may bring new dimensions to the identification power of HPLC. The detection limit of drugs depends on the efficiency of the isolation method applied and the spectral properties of particular compounds and ranges between 0.01 and 1 mg/l. These values are higher than those observed using UV spectrophotometric detectors. The problem of the sensitivity of detection seems to be a limiting factor at the moment, and therein lies the greatest potential for technical improvement.

4.4. Possibility of further expansion of database and exchange of data

The feasibility of standardization of retention data with the retention index scale applied made possible the interlaboratory use and expansion of the HPLC database. On the other hand, it should be stated that the standardization and the possibility of exchange of spectral data remain to be solved. Various manufacturers of diode-array detectors have developed different software systems that are not compatible and do not allow the transfer of spectral data from one library to another.

5. Abbreviations

A_1^1	absorbance of 1% solution in a 1-cm cuvette
ABP	(2-amino-5-bromphenyl)(pyridin-2-yl)methanone, hydrolysis product of bromazepam or 3-hydroxy-bromazepam
ACB	2-amino-5-chlorobenzophenone, hydrolysis product of chlordiazepoxide, clorazepate, demoxepam, nordiazepam, oxazepam or oxazolam
ACN	acidic/neutral drugs scale
ADB	2-amino-2′,5-dichlorbenzophenone, hydrolysis product of lorazepam
ANB	2-amino-5-nitrobenzophenone, hydrolysis product of nitrazepam
ANCB	2-amino-2′-chloro-5-nitrobenzophenone, hydrolysis product of clonazepam
ANFB	2-amino-2′-fluoro-5-nitrobenzophenone, hydrolysis product of norflunitrazepam
BAS	basic drugs scale
CCB	5-chloro-2-[(cyclopropylmethyl)amino]benzophenone, hydrolysis product of prazepam or 3-hydroxyprazepam
CCMK	1-cyclohexenyl 5-chloro-2-methylaminphenyl ketone, hydrolysis product of tetrazepam
DCFB	2-[2-(diethylamino)ethylamino]-5-chloro-2′-fluorobenzophenone, hydrolysis product of flurazepam
LSD	lysergic acid diethylamide
MACB	2-methylamino-5-chlorobenzophenone, hydrolysis product of camazepam, diazepam, ketazolam or temazepam
MAFB	5-amino-2′-fluor-2-(methylamino)-benzophenone, hydrolysis product of 7-aminoflunitrazepam
MATRAC	biological matrix peak in acidic extract
MATRBAS	biological matrix peak in basic extract

MDA	3,4-methylenedioxyamphetamine
MDB	2′,5-dichloro-2-(methylamino)-benzophenone, hydrolysis product of lormetazepam
MDE	3,4-methylenedioxyethylamphetamine
MDMA	3,4-methylenedioxymethylamphetamine
MNFB	2′-fluoro-2-(methylamino)-5-nitrobenzophenone, hydrolysis product of flunitrazepam
I	retention index
THC	tetrahydrocannabinol

6. Acknowledgements

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