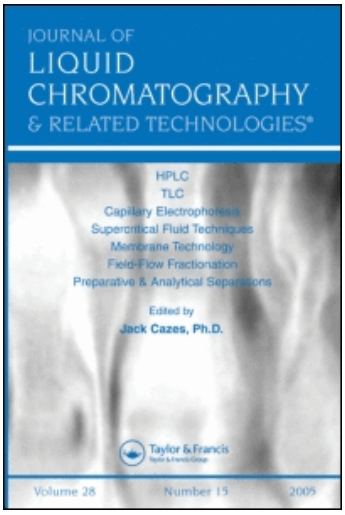

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Application of HPLC Equipment with Rapid Scan Detection to the Identification of Drugs in Toxicological Analysis

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APPLICATION OF HPLC EQUIPMENT WITH RAPID SCAN DETECTION TO THE IDENTIFICATION OF DRUGS IN TOXICOLOGICAL ANALYSIS

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ABSTRACT

The paper reports on an application of HPLC equipment with rapid scan detection, in which the UV-spectra recorded by the rapid scan detector are used for drug identification. A dBase database was set up, utilizing the retention data of a drug substance in a neutral and an acidic HPLC solvent together with the related UV spectrum. The identification of an unknown drug is supported by a home made search program, comparing the retention times first and then examining the maxima of UV spectra.
The data base presented here contains more than 200 entries, and data of additional substances are continuously attached.
It provides a fast and low cost possibility for the assay of drugs and medicaments in biological matrices and is especially used in emergency analysis.

INTRODUCTION

Much work has been done on the subject of assay and quantitative determination of drugs in biological matrices [1-3]. However, especially for the so called "general unknown analysis" there are still many problems left in the fields of forensic toxicology and analysis in emergencies. The increasing number of drugs to be encountered plays an important role in case of an intoxication with an unknown substance. During the last few years the number of available pharmaceutical products, narcotics, pesticides etc. has enormously increased. Therefore, a fast analysis leading to a reliable result has become a challenging procedure in case of intoxication.

HPLC analysis is well suited to meet these demands, and in case of a rapid scan detector the retention as well as the UV-spectrum can be used to identify a substance. Moreover, there are mathematical methods available for detecting peak overlays (proof of purity) and estimating spectra identities [4,5].

It is well known that it is very difficult to find the same retention data with measurements in different laboratories even in case of identical conditions. Therefore, it becomes necessary to develop home made data bases. This was done by us using the widespread dBase IV computer program, which now is available for a very low price.

The subject of this paper is to show that composing data sets from substances of toxicological relevance by combination of their retention data in two different eluents and the related UV spectra permits a sufficiently fast identification in toxicological analysis already by use of a home made dBase program. This low cost, but

high performance setup has been used by us since some month routinely in emergency analysis.

EXPERIMENTAL

Materials and Instrumentation

Two different isocratic HPLC setups (I and II) are used. Both of them, however, include an HPLC pump LC 1100 and an injection valve C6W from GAT(Gamma Analysentechnik Bremerhaven, Germany).
First, the setups differ in the detectors:

LC I: variable wavelength detector LCD 500 (GAT) at detection wavelength 254 nm, equipped with an C-R6A integrator (SHIMADZU)

and

LC II: rapid scan detector PHD 601 (GAT) at a detection wavelength range from 200 to 360 nm in steps of 1 nm; spectra are recorded by an IBM compatible personal computer with software delivered by the detector manufacturer.

The reference spectra were recorded from methanolic solutions of the substances.
A three-dimensional plot of the absorption as a function of wavelength and time as shown in Fig. 4 can be used to tune the detector for the highest sensitivity.
Second, the eluents for LC I and LC II are composed in the following way:

LC I : CH_3OH // 0.5 mol/l CH_3COOH //0.5 mol/l NH_4COOCH_3
(105 : 60 : 10 v/v/v) [6]

LC II: 156 g CH_3CN + 344 g phospate buffer solution pH=2.3
(4.8g 85 % H_3PO_4 and 6.66 g KH_2PO_4 in 1l H_2O)[7]
(All solvents: "BAKER ANALYZED" HPLC Reagent grade)

In both cases pre- (3.2 X 30mm) and main column (3.2 X 150 mm) are filled with ODS material (SI 100, 7 µm spheric), which was prepared in the former east German Central Institute of Organic Chemistry of the Academy of Sciences (Zentralinstitut für Organische Chemie der Akademie der Wissenschaften der DDR, ZIOC).
This material was investigated by EIGENDORF, who recommended it especially for the general unknown analysis [8].
The flow rate is to be chosen due to the retention time of the standard, normally between 0.5 and 1.0 ml/min.
Sample preparation is done by liquid-liquid extraction or solid phase extraction, the latter with Chem-Elut columns (Analytical-International) or Extrelut columns (MERCK).
From our experience, methanolic extracts from blood, urine or stomach contents are especially well suited for HPLC analysis.

Methods

Relative retention data were obtained by reducing the retention data of the investigated toxicological relevant substance to the retention time of 5-(4-Methylphenyl)-5-phenylhydantoin (MPPH) [9], which serves as standard substance.
UV spectra were recorded by LC II and stored on disk together with the chromatograms they were obtained from.

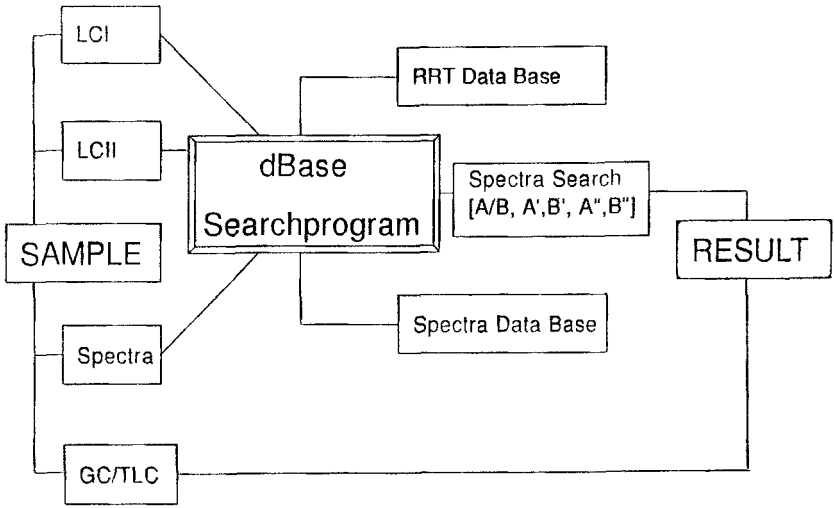

FIGURE 1: FLOW SCHEME OF SUBSTANCE IDENTIFICATION IN CASE OF AN ACCIDENTAL INTOXICATION

The chromatograms are used for the quantification of the unknown substance.

As can be seen from Figure 1 and mentioned before, access to the data base is provided by a home made dBase program [10], by which the following data of the references can be compared with the data of the unknown for identification:

A: relative retention data in LC I and LC II

B: relative retention data in LC II and spectra

C: relative retention data in LC I ,LC II and spectra

Concerning the identification by UV spectra [11-14], a pre-selection is done by comparing the UV maxima. The

TABLE 1
COMPILATION OF SOME OF THE RELATIVE RETENTION TIMES (RRT) AND ABSORPTION MAXIMA (AM) OF TOXICOLOGICAL RELEVANT SUBSTANCES AS MEASURED WITH SETUPS SPECIFIED IN THIS PAPER (LCI, LCII)

SUBSTANCE	LCI	LCII	AM1 [nm]	AM2 [nm]	AM3 [nm]	AM4 [nm]
Acetylsalicylsäure	0,39	0,47	231	298	0	0
Ajmalin	0,42	0,20	242	286	0	0
Aminophyllin	0,33	0,22	215	266	0	0
Bromhexin	1,53	0,48	208	242	311	0
Bromoprid	0,33	0,22	211	271	306	0
Butaperazin	0.27	0,46	238	273	0	0
Carbamazepin	0,78	0,58	208	233	282	0
Chinidin	0,44	0,17	207	245	312	340
Chlordiazepoxid	1,44	0,20	240	304	0	0
Chlorprothixen	2,07	0,82	202	225	264	324
Clenbuterol	0.00	0,24	207	240	295	0
Clonazepam	0,83	0,91	210	245	306	0
Coffein	0,37	0,21	225	267	0	0
Detajmium	0,29	0,15	240	284	0	0
Diazepam	1,94	1,58	227	279	310	0
Doxylamin	0,41	0,25	209	269	0	0
Ethosuximid	0,36	0,28	213	240	0	0
Flunitrazepam	0,87	1,19	213	248	308	0
Furosemid	0,43	0,64	227	266	338	0
Haloperidol	0,73	0,41	215	241	0	0
Hydrochlorothiazid	0,27	0,22	211	268	312	0
Levomepromazin	1,33	0,62	202	247	301	0
Metamizol	0,30	0,18	236	255	0	0
Methaqualon	1,00	0,92	222	262	301	313
Methocarbamol	0,40	0,27	218	269	0	0
Nicotin	0,28	0,09	254	0	0	0
Nitrazepam	0,87	0,69	211	255	303	0
Oxazepam	1,12	0,71	223	310	0	0
Parathionmethyl	1,90	4,44	270	0	0	0
Phenacetin	0,55	0,36	243	0	0	0
Phenazon	0,39	0,24	238	258	0	0
Phenprocoumon	1,89	4,07	280	305	0	0
Procain	0,42	0,20	216	290	0	0
Promazin	1,00	0,45	247	298	0	0
Propaphenon	1,00	0,52	204	244	300	0
Propranolol	0,63	0,28	210	225	286	312
Pyrithyldion	0,43	0,28	204	300	0	0
Salicylamid	0,40	0,33	230	296	0	0
Talinolol	0,57	0,26	238	282	0	0
Temazepam	1,25	1,18	226	308	0	0
Tetracain	0,62	0,33	222	309	0	0
Thebain	0,33	0,18	217	282	0	0
Thiopental	1,23	1,42	232	282	0	0
Triamteren	0,34	0,22	211	245	277	355
Verapamil	0,64	0,56	225	274	0	0
Zolpidem	0,44	0,24	203	233	292	0
Zopiclon	0,43	0,19	212	300	0	0
p-Nitrophenol	0,57	0,56	221	313	0	0

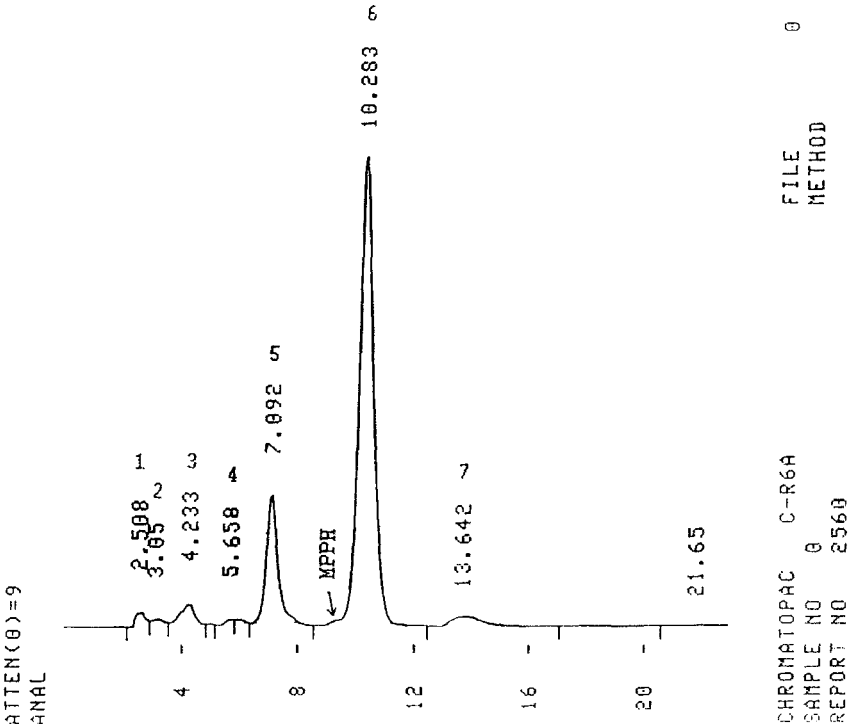

FIGURE 2: CHROMATOGRAM OF AN EXTRACT FROM SPE OF BLOOD IN AN EMERGENCY ANALYSIS (LC I, 220 nm). NOTE THE OVERLAY WITH THE STANDARD

range can be chosen, in which the experimental data must correspond to the stored data, i.e. a difference between the experimental and the stored spectrum of up to 9 % can be tolerated for the UV-spectra.

Once a number of substances are found similar to the unknown by the program, the further comparision is done by hand, inspecting the UV-spectra of the computer proposals for congruence with the spectrum of the unknown. During this step of manual search often also the first and second derivatives of the spectra are considered.

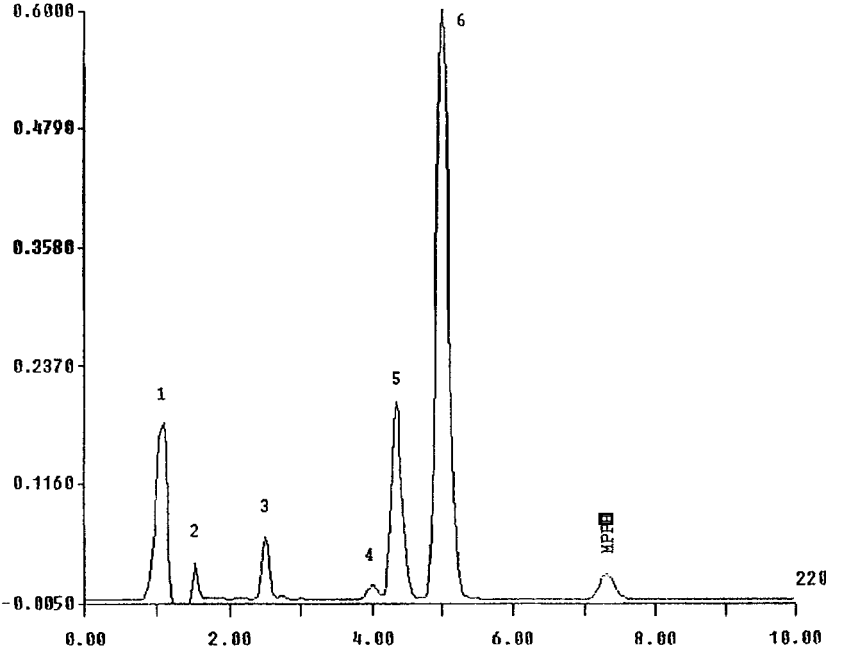

FIGURE 3: LC II CHROMATOGRAM OF AN EXTRACT OF SPE OF BLOOD IN AN EMERGENCY ANALYSIS (254 nm)

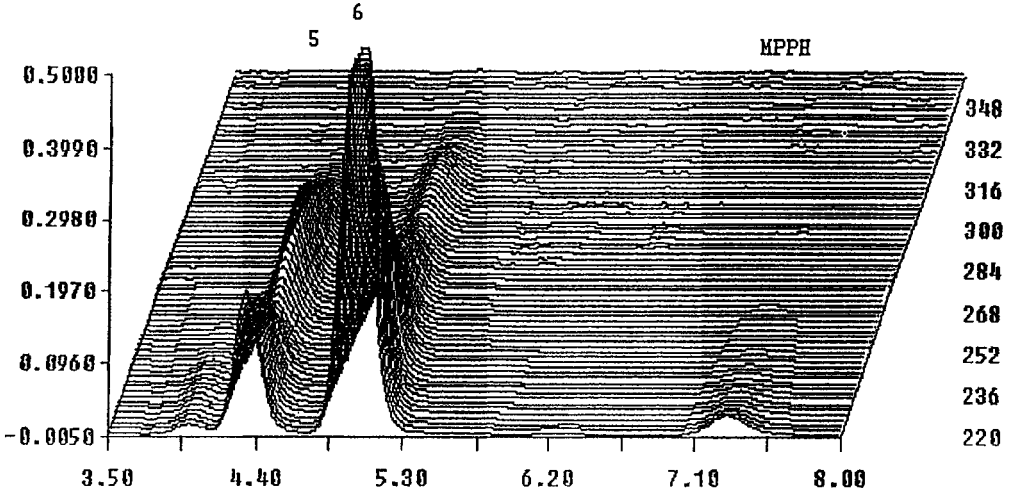

FIGURE 4: PART OF A THREE-DIMENSIONAL PLOT OF THE CHROMATOGRAPHIC INVESTIGATION OF THE BLOOD EXTRACT IN THE RAPID SCAN MODE (LC II)

YOUR INPUT WAS:

LCI	Tolerance [%]	from	to
0,26	10	0,23	0,29
0,32	10	0,29	0,35
0,44	10	0,40	0,48
0,60	10	0,54	0,66
0,74	10	0,67	0,81
1,08	10	0,97	1,19
1,43	10	1,29	1,57

YOUR INPUT WAS:

LCII	Tol. [%]	from	to	AM1 [nm]	from	to	AM2 [nm]	from	to	AM3 [nm]	from	to
0,15	10	0,14	0,17	219	210	228	275	266	284	0	0	0
0,21	10	0,19	0,23	235	226	244	0	0	0	0	0	0
0,34	10	0.31	0,37	0	0	0	0	0	0	0	0	0
0 54	10	0,49	0,59	235	226	244	0	0	0	0	0	0
0,59	10	0,53	0,65	208	199	217	235	226	244	280	271	289
0,69	10	0,62	0,76	225	216	234	310	301	319	0	0	0

SUBSTANCE	LCI	LCII	AM1 [nm]	AM2 [nm]	AM3 [nm]	AM4 [nm]
CARBAMAZEPIN	0,78	0,58	208	233	282	0
OXAZEPAM	1,12	0,71	223	310	0	0

FIGURE 5: RESULTS OF COMPUTER SEARCH BY THE dBase SEARCH PROGRAM

The results of the HPLC analysis should be validated by results of other methods (i.e. GC or TLC), whenever possible.

RESULTS AND DISCUSSION

Some of the relative retention data from LC I and LC II , as well as the absorption maxima in the range from 200 to 360 nm as obtained by rapid scan detection are shown in Table 1. The data base set up by us contains more than 200 data by now and will be continuously expanded.

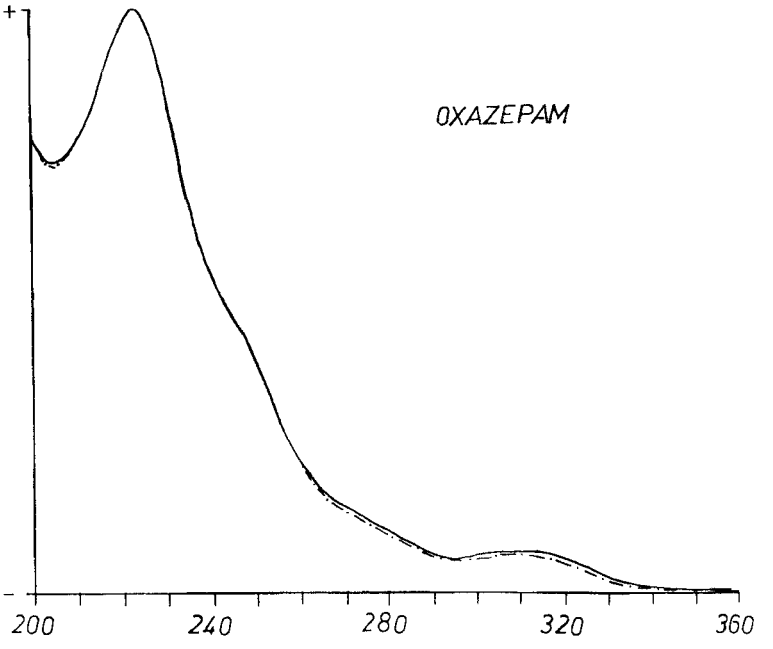

FIGURE 6: COMPARISON OF PEAK 6 (FIG. 3) WITH DATA BASE SPECTRUM FOR OXAZEPAME

The use of our method is now demonstrated by discussing an example of an analysis in an emergency:
A person was found unconscious with unknown cause. No indications for a special intoxication were reported. HPLC investigation of extracts from the persons blood resulted in the chromatograms given in Fig. 2 - Fig. 4. As mentioned before, the relative retention times and UV absorption maxima of the significant peaks were used for the computer search in the data base. Figure 5 shows the results of the search.
The proposal of the computer search could be verified by comparing the spectra as shown in Figure 6 and Figure 8,

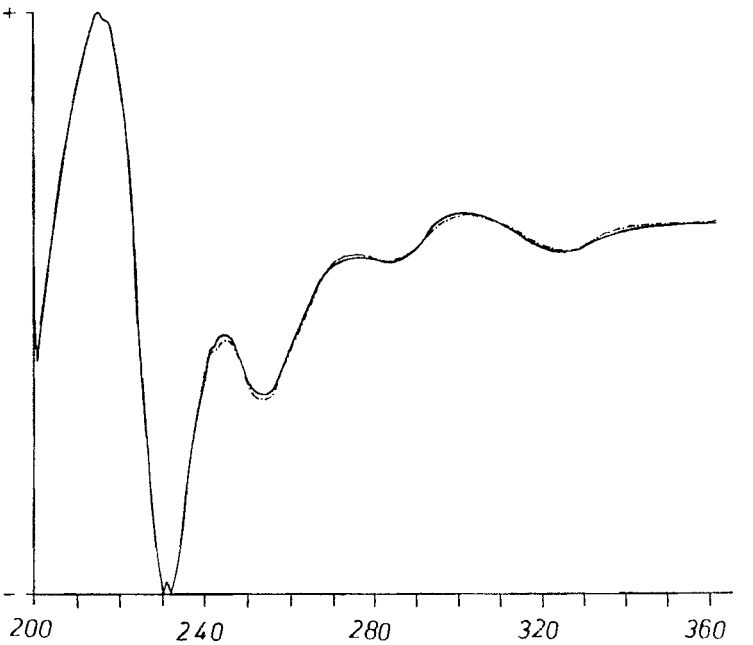

FIGURE 7: FIRST DERIVATIVE OF THE SPECTRA OF FIGURE 6

and the first derivatives of the spectra as shown in Figure 7 and Figure 9,respectively.

The excellent agreement of the spectra of the unknown substances with the substances proposed by the computer search is accomplished by the results of an enzymatic immunoassay (EMIT, Syva), which indicated the presence of benzodiazepines and tricyclic antidepressants.

Of course, the results are not always so unidirectional. Often about ten substances are included in the computer proposal, which have to be distinguished then by a more exhaustive spectra search. The HPLC investigation should anyhow be accomplished by another analytical method.

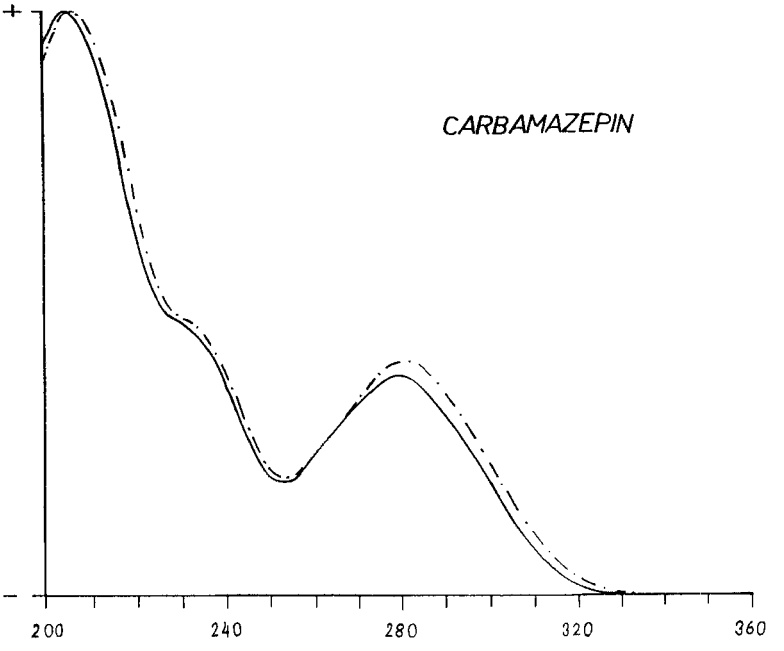

FIGURE 8: COMPARISON OF PEAK 5 (FIG. 3) WITH DATA BASE SPECTRUM FOR CARBAMAZEPINE

CONCLUSIONS

Provided that it is done by experienced personnel, HPLC emergency analysis as described above takes only about two to three hours, after which qualitative results are provided, having more than just temporarily character, because information from spectra are included. The method is fast and fairly simple, though some experience is needed. It can be highly recommended in those emergency cases, in which fast immunological drug screening does not suffice. It is amazingly less time consuming than classical methods of substance identification, which is of course especially meaningful in emergencies. Careful measurement of the absorption maxima of the references

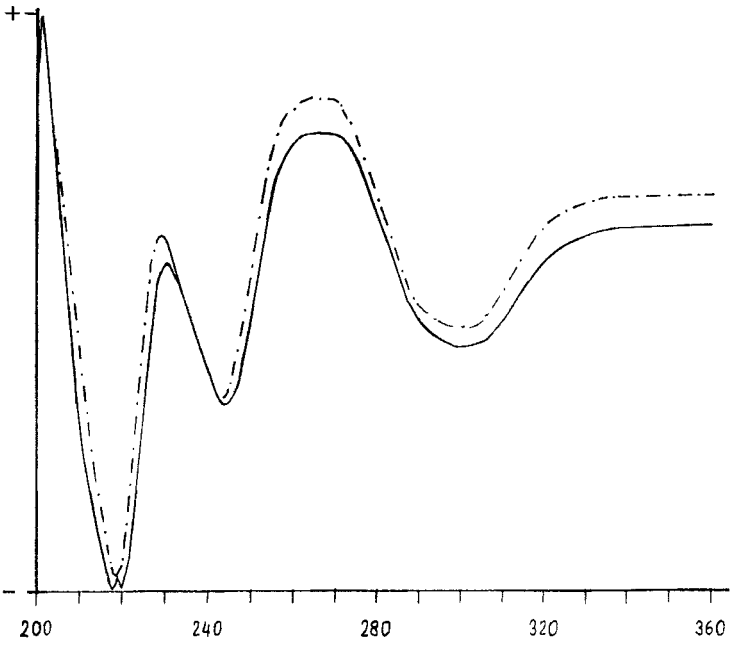

FIGURE 9: FIRST DERIVATIVE OF THE SPECTRA OF FIGURE 8

as well as the unknown substance is crucial to select reliable information from the data base.
There is of course more sophisticated equipment available (i.e. GC/MS, GC/HPLC). Considering limited fundings, however, the measurement of HPLC retention data and UV spectra by use of a rapid scan detector and data processing by use of standard software might be a low cost bypass, providing nearly the same information.

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