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# Determination of Dibenzepine in Autopsy Material

ROGER BONNICHSEN and BERTIL SCHUBERT
Government Laboratory for Forensic Chemistry, Stockholm (Sweden)

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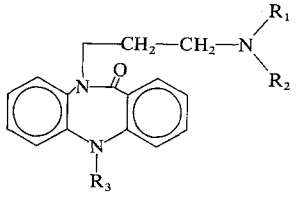
*Summary.* A method for detection and determination of dibenzepine in autopsy material is described. Since the introduction of this drug on the Swedish market in 1968 many fatal cases (probably all suicides) due to overdose of the drug have been discovered at our institute. The liver concentration of dibenzepine varies in our material from 0.2 to 33 mg per 100 g of tissue, and it seems that 2 to 5 mg of the non-metabolized drug per 100 g of liver represent a lower limit of lethal concentration in cases where no other drugs or ethanol are found.

*Zusammenfassung.* Eine Methode zu Entdeckung und Bestimmung von Dibenzepin in Leichenmaterial wird beschrieben. Tödliche Vergiftungen mit Dibenzepin, sämtliche wahrscheinlich Selbstmorde, sind in Schweden nicht selten, und die Analysen von 33 solchen Fällen sind hauptsächlich in Form von Tabellen präsentiert. Aus unserem Material scheint hervorzugehen, daß 2—5 mg nicht metaboliziertes Dibenzepin per 100 g Leber die untere Grenze einer tödlichen Konzentration darstellt in Fällen, wo andere Arzneimittel oder Äthanol nicht vorkommen.

*Key-Words:* Dibenzepine, Determination in Autopsy Material — Intoxication, Dibenzepine.




Dibenzepine-5-methyl-10-$\beta$-dimethylaminoethyl-10,11-dihydro-11-oxo-5H-dibenzo[b,e][1,44]diazepine-($R_1$,$R_2$,$R_3 = CH_3$) and its demethylated metabolites. Designation of the metabolites are according to [8].

| Metabolite | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| II | H | $CH_3$ | $CH_3$ |
| III | $CH_3$ | $CH_3$ | H |
| IV | H | $CH_3$ | H |
| V | H | H | $CH_3$ |
| VI | H | H | H |

The synthesis of dibenzepine was described by Hunziker *et al.* in 1963 [1]. It was later introduced on the market as an antidepressive drug "Noveril"®, and since 1968 it is marketed in Sweden under the name of Neodalit®. Fatal

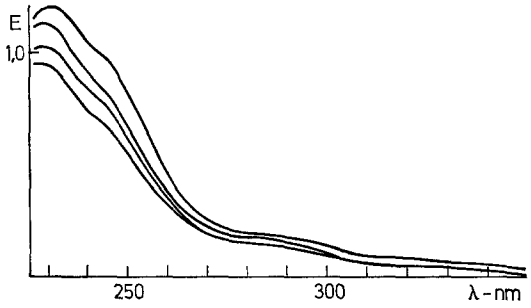



Fig. 1. Ultra-violet absorption spectra of (from the top): 1. a solution of dibenzepine, 20 μg/ml; 2. an "alkaline" extract from the liver of a poisoning case; 3. a similar extract from the same case, where the tissue sample was hydrolyzed prior to extraction; 4. a "neutral" extract from the same case, according to the procedure used for screening of hypnotic drugs (p. 254). The recordings were performed with a Unicam SP-800 instrument with 10 mm cuvets. The samples were dissolved in 0.025 N ammonia in 75% ethanol. Recordings 2–4 correspond to 0.04 g of liver per ml solution

poisonings with dibenzepine have been described by Reinhardt *et al.* [2] and by Brochon *et al.* [3]. In this paper a number of unexpected deaths are presented where dibenzepine alone or together with other drugs was found at the chemical analyses.

## Material and Methods

The tissue examined originated from autopsies performed at various institutes of forensic medicine in Sweden. Pure dibenzepine was obtained by extracting Neodalit® tablets and recrystallizing the residue from acetone-water. The compounds II—VI were supplied by AB Hässle, Gothenburg, Sweden. The chloroform used was of technical grade and redistilled over anhydrous calcium chloride prior to use.

When tissue samples are analyzed according to the routine method employed at the laboratory for determination of barbiturates and some other drugs (Bonnichsen [4], cit. fr. [5]), dibenzepine concentrations exceeding 0.4 mg per 100 g of tissue are readily observed by ultra-violet spectrophotometry of the "neutral" extract (see Fig. 1). The spectrum is not very specific and does not allow an accurate quantitative determination. If the presence of dibenzepine thus is suspected, as a rule, another aliquot of the ethanol extract (corresponding to 10 g of tissue) is evaporated to a small volume, water is added to a total volume of 25 ml and the solution alkalinized with 25% ammonia to about pH 11.

The further extraction is then carried out as for phenothiazine drugs [6], the hydrolysis step being omitted. Ultra-violet spectrophotometry and thin layer chromatography is performed according to the last mentioned paper and finally aliquots of the extracts are analyzed by gas chromatography. The concentration of dibenzepine in the sample is calculated from the peak heights on the recorder.

## Results and Discussion

To our knowledge, no figures have been published about the concentrations of dibenzepine in blood and urine from patients treated with the drug. After administration of the radioactive drug to mice and rabbits, the gall bladder, the liver and the kidney showed the greatest activity [7]. In man (autopsy material) and some animals, the drug was found to be eliminated through the urine mostly in metabolized form, human urine contained all the possible demethylated com-

pounds [8]. Reinhard *et al.* [2] demonstrated the presence of dibenzepine in material from two autopsy cases. No quantitative determinations were carried out. Brochon *et al.* [3] investigated material from three autopsies. They employed various methods of extraction and got the highest yield by extracting the tissue homogenate with hot hydrochloric acid (about 4 N). Somewhat lower values were obtained with direct extraction of the homogenates with organic solvents, whereas the use of protein precipitating agents resulted in extensive losses of dibenzepine. Quantitative determination was performed by gas chromatography according to Lehner *et al.* [8], and values were given for dibenzepine and metabolite II, other metabolites being present only in minor quantities. The metabolite content in blood, liver, brain and kidney were found to be lower than that of the parent drug. In our investigation, gas chromatographic determinations were performed in some autopsy cases on solutions from three different extraction methods of the same sample. Two of them are described under "Materials and methods", and the third type of extraction was done with hydrochloric acid according to Brochon *et al.* [3]. Determination of the unaltered dibenzepine in the three extracts by gas chromatography gave approximately the same result, the last mentioned procedure giving a somewhat higher yield. Alkaline hydrolysis of the tissue prior to extraction [6] did not significantly increase the yield of dibenzepine. A typical gas chromatographic recording is presented in Fig. 2. In this system no metabolites of the drug were found to interfere with the determination of dibenzepine.

The specificity of the method was proved in one case (Table 1, no. 18) by infrared spectrophotometry. The gas chromatographic effluent, with a retention time corresponding to that of dibenzepine, was collected through a splitter, and its IR-spectrum agreed well with that of pure dibenzepine (Fig. 3).

Values from all the cases investigated are presented in Table 1. The figures are based upon gas chromatographic determinations (with a few exceptions noted in the foot-notes of Table 1) and thus involve only unaltered dibenzepine. When available data about the probable dose ingested and certain other observations are included in the table. Remarks concerning autopsy findings are based upon the medical examiners' reports from the gross post mortem examination.

Samples from the cases in Table 1 were also analyzed for certain other drugs as barbiturates, salicylates, meprobamate, methaqualone and various tricyclic amines. When alcohol was suspected also blood alcohol determinations were made. The results are listed in the last column of the table.

Striking features of the cases in Table 1 are that a great number of young persons are included, and further that alcohol misuse seems to be rare (only two cases show an apparent fatty degeneration of the liver). This is in contrast to the average picture of suicide victims.

Table 2 show the relative distribution of fatal poisonings with dibenzepine and related antidepressive drugs. The high relative frequency already in 1969 of dibenzepine seems remarkable. From Table 1 it is very difficult to draw any certain conclusions about the lower limit for lethal concentrations of dibenzepine in the liver. It seems that this limit might lie at about 2 to 5 mg per 100 g of liver, as cases with lower amounts probably died from a combination of drugs or secondary complications e.g. pneumonia. In many instances tablets were found in the stomach, death thus occurring before resorption of all the dibenzepine.

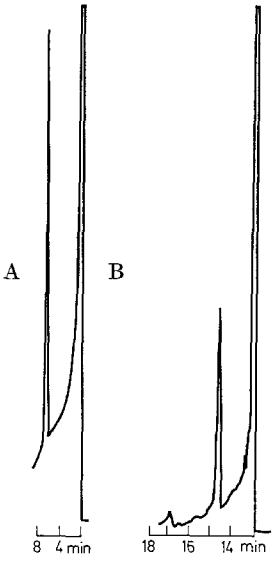



Fig. 2 A and B

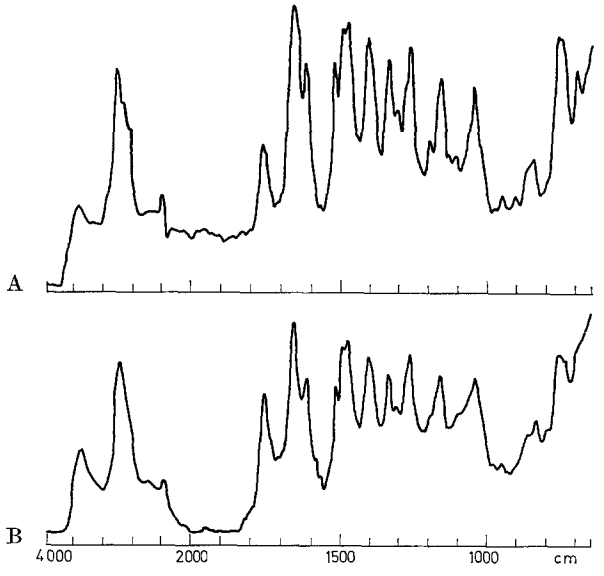



Fig. 3 A and B

Fig. 2. A, shows a gas chromatographic recording of dibenzepine solution in chloroform (0.40 μg of the drug was injected). B, shows a recording from extract of liver (the amount injected corresponds to 8 mg of liver). Conditions: Pye 104 gas chromatograph was used with a glass column 1.5 m × 3 mm (I.D.) filled with 1% HiEff 8 BP on Gas Chrom Q. The temperature of the column was 220°, that of the detector and injector 260°. The flowrates of nitrogen (carrier gas) and hydrogen (to the F.I.D.) were about 50 ml/min. Attenuation was 500, recording paper rate 2.5 mm/min

Fig. 3 A and B. Infra-red absorption spectra of (A) pure dibenzepine, (B) eluate from the gas chromatographic separation of liver extract from case No. 18 (Table 1) (compound with the same retention time as dibenzepine). Conditions: the recordings were performed with a Hilger H 800 apparatus, equipped with a beam condensor. The samples were deposited on KBr discs

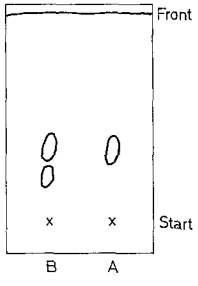



Fig. 4. Thin-layer chromatography of (A) 25 μg dibenzepine, (B) extract from liver. The amount applicated to the starting point corresponds to 1 g of liver. Ready-made TLC plates were used (DC-Alufolien, Kiselgel F2 54 from Merck AG, Germany) and the developing solvent was methanol—25% ammonia (100+2). The areas marked are ultra-violet absorbing and turn orange-red after spraying with Dragendorff's reagent

In animal experiments [8] dibenzepine underwent enterohepatic circulation, which probably complicates the interpretation of the analytical data.

Thin-layer chromatography of the extracts was performed as described for other tricyclic antidepressive amines [6, 9]. In addition to dibenzepine, one or sometimes two or three spots were observed that absorb ultra-violet light and turn orange-red with Dragendorff's reagent (Fig. 4). Absorption spectra of eluted

Table 1. *Dibenzepine concentrations found in 33 autopsy cases. The figures are calculated from gas chromatography recordings (thus referring to the unaltered drug), unless otherwise noted. The cases are presented in the order of increasing liver concentration of dibenzepine (no liver samples were available for analysis in case no. 31–33). The concentrations of other drugs found and of ethanol are also given*

| No. | Sex | Age | Notes about suicide, etc. | Tablet intake; course of intoxication | Remarkable autopsy findings | Analytical findings (mg/100 g; EtOH: %) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Dibenzepine | | Other drugs or EtOH | | |
| | | | | | | Liver | Blood | Liver | Blood | |
| 1 | f | 24 | earlier suicide attempt | T, died 8 h a.t.i. in convulsions | 0 | 0.2[a] | — | 0 | 0 | |
| 2 | m | 28 | earlier suicide attempt | 100 tablets; coma 45 min a.t.i.; T | aspiration of stomach contents | 0.3 | — | 0 | 0 | |
| 3 | m | 29 | suicide; alcoholic | T (1 day) | tablets in stomach | 0.8 | — | 0.8 | 0.7 | glute-thimide |
| 4 | f | 13 | suicide? psychotic | 20(?) tablets; 10 mg diazepam intravenously | pneumonia | 1.0[a] | 0.3[b] | 0 | 0[c] | |
| 5 | f | 18 | suicide; depressio | coma 2.5 h a.t.i., T | slight aspiration of stomach contents | 1.1[a] | — | 0 | 0 | |
| 6 | f | 26 | earlier suicide attempt; day before death slight overdose of diazepam | T; convulsions finally | tablets in stomach | 1.4 | 0.5 | 0 | 0 | |
| 7 | f | 19 | — | found dead | 0 | 1.5[a] | 0.1[d] | 0 | 0 | |
| 8 | f | 17 | — | rapid course, convulsions; T | tablets in stomach | 1.8 | — | 0 | 3.5 | metro-nidazol |
| 9 | f | 17 | suicide? depressio | dead after 10 h | tablets in stomach | 2.5 | 0.7[e] | 0 | 0 | |
| 10 | f | 22 | depressio | 40(?) tablets; T | tablets in stomach | 2.5[a] | 0.5 | 0 | 0 | |
| 11 | f | 26 | suicide | found dead | tablets in stomach | 3.0 | — | 0 | 0.01 | EtOH |
| 12 | f | 43 | suicide; earlier drug abuse | found dead | tablets in stomach | 3.5[a] | — | 0 | 0 | |
| 13 | m | 33 | suicide; alcoholic | found dead | 0 | 3.8 | —[f] | 0 | 0.12 | EtOH |
| 14 | m | 28 | suicide | 100 tablets; found dead | tablets in stomach and bronchi | 4.0 | — | 0 | 0.11 | EtOH |
| 15 | m | 28 | suicide | found dead | tablets in stomach | 5[g] | — | 0 | 0.02 | EtOH |

Table 1 (Continued)

| No. | Sex | Age | Notes about suicide, etc. | Tablet intake; course of intoxication | Remarkable autopsy findings | Analytical findings (mg/100 g; EtOH: %) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Dibenzepine | | Other drugs or EtOH | | |
| | | | | | | Liver | Blood | Liver | Blood | |
| 16 | f | 26 | misuser of drugs | died about 4 h a.t.i., T | 0 | 5.3 | — | 0 | 0 | |
| 17 | f | 57 | depressio | — | tablets in stomach; coronary arteriosclerosis | 6.2 | — | 0 | 0 | |
| 18 | m | 47 | alcoholic | found dead | fatty liver | 6.3 | — | 0 | 0.27 | EtOH |
| 19 | m | 19 | possible nacrotic | found dead | tablets in stomach; probably aspiration | 6.6 | — | 0 | 0 | |
| 20 | m | 23 | — | found dead | — | 7.3 | — | 0 | 0.07 | EtOH |
| 21 | m | 28 | suicide; formerly drug abuser | found dead | tablets in stomach | 8.7 | — | 0 | 0 | |
| 22 | f | 39 | suicide; psychotic | convulions finally | tablets in stomach | 9.4 | — | 0 | 0 | |
| 23 | f | 27 | suicide | dead about 2 h a.t.i. | aspiration of stomach contents | 12.3 | — | 0 | 0.01 | EtOH |
| 24 | f | 16 | cannabis smoker; earlier suicide attempt | 30 (?) tablets; convulsions before death | slight aspiration of stomach contents; tablets in stomach | 12.5 | — | 0 | 0 | |
| 25 | m | 24 | suicide | 100 (?) tablets; found dead | — | 14 | 1.9 | 0 | — | |
| 26 | f | 33 | suicide | 80 tablets; found dead | 0 | 14 | — | 0 | 0 | |
| 27 | m | 27 | suicide; mental illness | 100 tablets | tablets in stomach | 15[g] | — | 0 | 0 | |
| 28 | m | 34 | earlier suicide attempts | found dead; drowned in the bath-tube | tablets in stomach | 15 | — | 0 | 0.03 | EtOH |
| 29 | f | 50 | — | — | 0 | 22 | — | 0 | 0 | |
| 30 | m | 52 | suicide; depression | found dead | tablets in stomach | 33 | — | 3.0 | — | amitriptyline |
| 31 | f | 33 | — | — | — | — | 0.3 | 0.06 | — | amphetamine |

Table 1 (Continued)

| No. | Sex | Age | Notes about suicide, etc. | Tablet intake; course of intoxication | Remarkable autopsy findings | Analytical findings (mg/100 g; EtOH: %) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Dibenzepine | | Other drugs or EtOH | |
| | | | | | | Liver | Blood | Liver | Blood |
| 32 | m | 21 | suicide | — | — | —[h] | — | — | 0.06[i] oxazepa |
| 33 | f | 43 | suicide | found dead | tablets in stomach and duodenum | —[j] | 1.1 | — | 0.07 EtOH |

— = no information or not determined. 0 = no significant findings or not found. T = treated in hospital before death. a.t.i. = after tablet intake. EtOH = ethanol.

[a] UV-spectrum and/or TLC indicated the presence of great amounts of metabolites (equal to or exceeding the concentration of unaltered drug).

[b] Kidney: 0.7.

[c] Diazepam: under the limit of detection.

[d] Urine: 1.8.

[e] Urine: 17; kidney: 2.3.

[f] Urine: 11; kidney: 4.0.

[g] Value, estimated from TLC.

[h] Urine: 1.3.

[i] Urine: 2.8.

[j] Kidney: 8.8; urine: 1.8.

Table 2. *Autopsy cases analyzed at the Government Laboratory for Forensic Chemistry in Sweden, where dibenzepine and other tricyclic antidepressive drugs*[a] *were found, in the period Aug., 1968—June, 1970. Only cases with 0.5 mg or more per 100 g of liver are included. "Combined" are those poisoning cases, where also other drugs and /or ethanol (0.05% or more in blood) were detected*

| Period | No of cases | | | | | |
|---|---|---|---|---|---|---|
| | dibenzepine | | amitriptyline/ nortriptyline | | other anti-depressives | |
| | alone | combined | alone | combined | alone | combined |
| 1.8.—31.12.1968 | 1 | 0 | 2 | 7 | 0 | 1 |
| 1.1.—31.12.1969 | 14 | 5 | 7 | 11 | 4 | 4 |
| 1.1.—30. 6.1970 | 6 | 1 | 7 | 5 | 3 | 7 |

[a] Imipramine, trimipramine, opipramol, desipramine, amitriptyline, nortriptyline and protriptyline.

metabolite spots were similar to those of pure dibenzepine and of compounds II—VI. In some cases the metabolite concentration in liver seemed to considerably exceed that of the parent drug judged from the thin-layer plate. In such cases the presence of great amounts of metabolites was also indicated by the ultra-violet spectrum (Fig. 1), which showed the presence of much more material than did the gas chromatographic peak that corresponds to unaltered dibenzepine. Identification of the various metabolites has not yet been undertaken, and in some cases with a rapid fatal course no metabolites were detected.

## References

1. Hunziker, F., Lauener, H., Schmutz, J.: Zur Chemie und Pharmakologie von in 10-Stellung basisch substituierten 5-Dibenzo-[b,e][1,4]-diazepin-Derivaten. Arzneimittel-Forsch. **13**, 324–328 (1963).
2. Reinhardt, G., Geldmacher, J. H., Geldmacher-v. Mallinckrodt, M.: Zur Toxicität von Noveril. Nervenarzt **39**, 473–476 (1968).
3. Brochon, R., Lehner, H., Gauch, R., Rudin, O.: The detection and determination of dibenzepine and its metabolites in autopsy material. Arch. Toxikol. **24**, 249–259 (1969).
4. Bonnichsen, R.: Personal communication (1970).
5. Frøslie, A., Schubert, B.: Analysis of methaqualone in autopsy material. Z. Rechtsmedizin **67**, 342–358 (1970).
6. Bonnichsen, B., Geertinger, P., Maehly, A. C.: Toxicological data on phenothiazine drugs in autopsy cases. Z. Rechtsmedizin **67**, 158–169 (1970).
7. Michaelis, W.: Zum Stoffwechsel von 5-Methyl-10-$\beta$-dimethyl-aminoäthyl-10,11-dihydro-11-oxo-5H-dibenzo-[b,e][1,4]-diazepin. HCl. II. Mitt. Arzneimittel-Forsch. **17**, 181–185 (1967).
8. Lehner, H., Gauch, R., Michaelis, W.: Zum Stoffwechsel von 5-Methyl-10-b-dimethyl-aminoäthyl-10,11-dihydro-11-oxo-5H-dibenzo-[b,e][1,4]-diazepin. HCl. III. Mitt. Arzneimittel-Forsch. **17**, 185–189 (1967).
9. Bonnichsen, R., Maehly, A. C., Sköld, G.: A report on autopsy cases involving amitroptyline and nortriptyline. Z. Rechtsmedizin **67**, 190–200 (1970).

Professor Dr. Roger Bonnichsen
Dr. Bertil Schubert
Statens rättskemiska laboratorium
S-10401 Stockholm 60