

Detection of Drugs other than Barbiturates in the Routine Method for Barbiturate Analysis

A. C. MAEHLY and M. K. LINTURI

Statens Rättskemiska Laboratorium, Stockholm 60, Sweden*

When barbiturates are extracted from autopsy tissue, a number of other toxicologically important compounds are extracted at the same time. This paper presents data obtained by paper chromatography and spectrophotometry for more than 20 of these compounds.

In a recent publication¹ we described the isolation, identification, and quantitative determination of barbiturates from human tissue. Several thousand extractions of tissue, blood and urine from autopsy cases have been carried out according to this method. The procedure in brief is as follows:

The tissue (5–50 g) is ground in a blender (blood or urine is of course taken as such), acidified to pH 3–4 with dilute hydrochloric acid, and extracted with chloroform. The solvent is evaporated and the residue treated with hot dilute hydrochloric acid. The solution is cooled to +4°, fat removed by filtration, and the filtrate reextracted with chloroform. This extract is brought to a known volume (usually 5.0 ml) and aliquots analyzed by paper chromatography and spectrophotometry.

It has been found that quite a number of neutral and acidic compounds of toxicological importance, other than barbiturates, can be detected and assayed at the same time¹. The advantages of extracting such a variety of drugs in one and the same procedure are obvious. In order to facilitate further analysis it is advisable to separate acidic compounds (barbiturates, salicylic acid, sulfonamides) from the chloroform extract mentioned above and to assay the acidic and the neutral substances separately. To this end we use the following simple procedure:

The chloroform extract (usually 5 ml) is shaken four times with an equal volume of 0.5 N ammonia, separating the solvent layers by centrifugation. The aqueous layers are combined, acidified with hydrochloric acid, and reextracted three times with twice their volume of chloroform. The combined chloroform extracts (containing the acidic compounds) as well as the original chloroform solution (retaining the neutral substances) are each dried over sodium sulfate, filtered, and brought to a known volume, usually 5.0 ml.

* The Government Laboratory for Forensic Chemistry.

Table 1. R_F -values of some non-barbiturate drugs.

Compound		R_F -values			Visibility on the paper
Merck Index name (where possible)	Synonym	diethyl ether	di- <i>n</i> -butyl ether **	chloroform	
<i>p</i> -Acetaminophenol	NAPA	0.30	0	0	UV
Acetophenetidine *	Phenacetine	0.90	0.55	0.95	UV
Acetylcarbromal	Abasin	0.87	0.82	0.93	(a)
Aminopyrine	Pyramidon	0.82	0.77	0.94	UV
Antipyrine *	Phenazone	0.55	0.11	0.94	UV, (c)
Bemegride	Megimide	0.91	Fr	Fr	(a)
Bromisovalum	Bromural	0.90	0.84	0.89	(b)
<i>c</i> -Bromovalerylurea	Bromyl	0.90	0.89	0.89	(b)
Caffeine *	—	0.24	0.09	0.95	UV
Carbromal	Adalin	0.87	0.88	0.92	(a)
Dextromoramide	Palfium	Fr	Fr	Fr	(c)
5-Ethyl-3-methyl-5-phenylhydantoin	—	—	—	—	(a)
Glutethimide	Difhydan	0.80	0.20	0.56	(a)
Meprobamate	Doriden	0.90	0.82	0.95	(d)
Phenothiazine derivatives	—	0.80	0.19	0.74	(e)
Salicylamide	<i>e.g.</i> , Lergigan, Chlorpromazine	Fr	Fr	Fr	UV, (c)
Salicylic acid	—	0.25	0.07	0.56	UV, F
Sulfamethazine	—	0	0	0	UV, F
Sulfathiazole	Sulfadimidin	0.02	0	0.07	UV
Theobromine	—	0	0	0	UV
Theophylline	—	0	0	0.22	UV
Truxal	—	0	0	0.15	UV
	<i>Trans</i> -2-chloro-10-(3-dimethylamino-propyliden) thia-xanthene acetate	Fr	Fr	Fr	UV

The following symbols are used:

UV Visible on illumination with ultraviolet light (Hg-line at 254 $m\mu$) when a fluorescent paper is placed under the paper chromatogram as described elsewhere ¹. The compound is listed in Table 2.

F The compound fluoresces on illumination with UV-light.

* The chromatographic and spectral properties of these compounds have been described previously ³.

** Running time 5 h; the solvent front has then left the paper.

Fr At the solvent front.

a) Only large amounts (*ca.* 0.5 mg and more) are visible in UV-light (Hg-line at 254 $m\mu$).

b) Only amounts larger than 0.1 mg are visible in UV-light (Hg-line at 254 $m\mu$).

c) Gives a positive reaction with potassium bismuth iodide (Dragendorff's reagent), described, *e.g.*, by Curry ⁴.

d) Only visible in UV-light if more than 1 mg is present. A color reaction for glutethimide, using mercurous nitrate, has been described by Dressler ⁵.

e) Meprobamate reacts with the chlorine-benzidine reagent described by Vidic ⁶.

Table 1 shows the R_F -values obtained by using 3 different, water-saturated solvents, *i.e.* diethyl ether, di-*n*-butyl ether and chloroform. Whatman No. 1 paper, previously treated with sodium carbonate solution, and the descend-

ing technique was used. In Table 2 the compounds exhibiting absorption maxima in the ultraviolet are arranged in the order of increasing wavelength of their maxima. The extinction coefficients for 1 % (w/v) solutions, using 1 cm lightpath are also given *. Drugs with distinct absorption peaks can not only be detected but also assayed quantitatively. The compounds lacking

Table 2. Ultraviolet absorption maxima of compounds that are extracted together with barbiturates.

1. *Wavelengths* (λ) in $m\mu$. Accuracy $\pm 2 m\mu$. 2. *Names of compounds*: If possible, the name listed in the »Merck Index», 7th ed., is given. In some cases other names are listed as well. 3. *Solvents*: a-EtOH: ammoniacal 75 % ethanol; pH on diluting with an equal volume of water *ca.* 10. s-EtOH: acidified 75 % ethanol; pH after dilution *ca.* 2. NH_4OH : 0.5 N ammonium hydroxide. H_2SO_4 : *ca.* 0.01 N sulfuric acid. KOH: *ca.* 0.1 N potassium hydroxide. 4. *Extinction coefficients*: for 1 % (w/v) solutions, measured in a cuvet with 1.0 cm optical pathlength ($E_{cm}^{1\%}$). Accuracy ± 3 % except where denoted by »*ca.*».

Absorption maxima ($m\mu$)		Compound	Solvent	$E_{1cm}^{1\%}$
maxi- mum	other maxima			
228		Bemegrade (Megimide)	NH_4OH	470
228†	(296)	Salicylic acid	a-EtOH	488
230*	(268, 325)	Truxal (Lundbeck) ^a	{ a-EtOH s-EtOH	287
233†	(300)	Salicylic acid	s-EtOH	558
235†	(302)	Salicylamide	s-EtOH	630
237	(286)	Thiobarbiturates	H_2SO_4	<i>ca.</i> 340
240		Barbiturates	NH_4OH	<i>ca.</i> 400
242		Br-substituted barbiturates	NH_4OH	<i>ca.</i> 400
242	(260)	Sulfamethazine	a-EtOH	730
244		N-alkylated barbiturates	{ NH_4OH KOH	<i>ca.</i> 400
244*	(270)	Antipyrine (Phenazone)	{ a-EtOH s-EtOH	488
245		Sulfadimethoxine	s-EtOH	350
246		Barbiturates that are Br-substituted and N-alkylated	{ NH_4OH KOH	<i>ca.</i> 400
247		5-Hydroxyethyl-4-methyl-thiazole	a-EtOH	<i>ca.</i> 160
248		<i>p</i> -Acetaminophenol (NAPA)	{ a-EtOH s-EtOH	853
250		Acetophenetidine (Phenacetine)	{ a-EtOH s-EtOH	822
252*		Promethazine (Lergigan)	s-EtOH	955
255*		Promethazine	a-EtOH	915
255		5-Hydroxyethyl-4-methyl-thiazole	s-EtOH	<i>ca.</i> 160
255		Barbiturates	KOH	<i>ca.</i> 300
255*		Chloropromazine	s-EtOH	1040
256*		Chloropromazine	a-EtOH	990

a) *Trans*-2-chloro-10(3-dimethylaminopropyliden)-thioxanthene acetate.

* A similar list has been published in other connections by Bradford and Bracket ⁷.

Table 2. Continued.

256		Sulfathiazole	NH ₄ OH	256
256	(304)	Thiobarbiturates	NH ₄ OH	ca. 400
257		Br-substituted barbiturates	KOH	ca. 300
260	(242)	Sulfamethazine	a-EtOH	740
262		Aminopyrine (Pyramidon)	a-EtOH	406
268*	(230, 325)	Truxal ^a	{s-EtOH	122
			{a-EtOH	
270		Theophylline	a-EtOH	664
270*	(244)	Antipyrine	{s-EtOH	480
		(Fenazon)	{a-EtOH	
271		Caffeine	s-EtOH	400
272		Caffeine	a-EtOH	420
272		Theobromine	s-EtOH	406
274		Theophylline	a-EtOH	640
275		Theobromine	a-EtOH	550
280		Sulfathiazole	H ₂ SO ₄	180
286	(237)	Thiobarbiturates	H ₂ SO ₄	ca. 900
296†	(228)	Salicylic acid	a-EtOH	290
300†	(233)	Salicylic acid	s-EtOH	305
302†	(235)	Salicylamide	s-EtOH	310
304	(256)	Thiobarbiturates	NH ₄ OH	ca. 1160
325*	(230, 268)	Truxal ^a	{s-EtOH	28
			{a-EtOH	
332†	(235)	Salicylamide	a-EtOH	440

† Fluoresces on the paper.

* Gives a positive reaction with Dragendorff's reagent (potassium bismuth iodide).

such spectral characteristics must be assayed in other ways. For some of them, quantitative methods are available; investigations on the quantitative determination of others are in progress.

Ultraviolet spectra and R_F -values are usually not sufficient for the certain identification of compounds of toxicological importance. A method for identification of micro amounts of compounds that can be sublimated *in vacuo* for both infrared spectrophotometry and melting point determination is described elsewhere ².

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