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The Analysis of Meperidine and Normeperidine in Biological Specimens

This study centers on the analytical aspects of meperidine analysis in biological specimens. The major meperidine metabolite normeperidine is treated concomitantly throughout as it is considered important in the interpretation of results in medical examiner/coroner cases and in overdosed patients. Previous studies have presented methods for the gas chromatographic analysis of meperidine in blood, plasma, or urine [*1-3*]. Other reports have presented procedures for meperidine and normeperidine [*4-7*] but did not deal with tissue analysis. Kazyak [*8*] reported combined meperidine and normeperidine in blood, urine, and four tissues but did not analyze the two compounds separately. A gas chromatographic method for meperidine and normeperidine in blood, urine, bile, liver, and other tissues is presented here. The method is straightforward with no derivatizing reactions prior to gas chromatography (GC); analysis time for liver is about 1 h, and blood and urine can be analyzed in 30 min. Results from twelve medical examiner cases are presented and discussed.

Methods

Extraction Techniques

Blood, urine, and other aqueous fluids were extracted as diagramed in Fig. 1. Ethyl ether/*n*-hexane/isopropanol (4:1:0.1, v/v), five volumes solvent to one volume of aqueous phase, was found to be effective in removing meperidine, normeperidine, and mepivacaine (the internal standard) from blood, bile, or urine, and two half-volume extractions with dichloromethane/isopropanol (10:1) removed these bases from alkaline aqueous solution in the back extraction step. The use of dichloromethane rather than chloroform in the final extraction step is necessary to prevent formation of an artifact produced from normeperidine and ethyl chloroformate, a contaminant present in chloroform. As we reported [*9*], the artifact produced is normeperidine ethylcarbamate.

Liver was processed in the manner shown in Fig. 2. Better drug recoveries were obtained for meperidine and normeperidine by initial protein precipitation with ammonium sulfate-hydrochloric acid rather than by extracting tissue homogenate directly with the extraction solvent mixture. Relative to direct solvent extraction, the protein precipitation yield was doubled and the yield of normeperidine increased by about one third. This comparison was made with methyprylon as an external standard.

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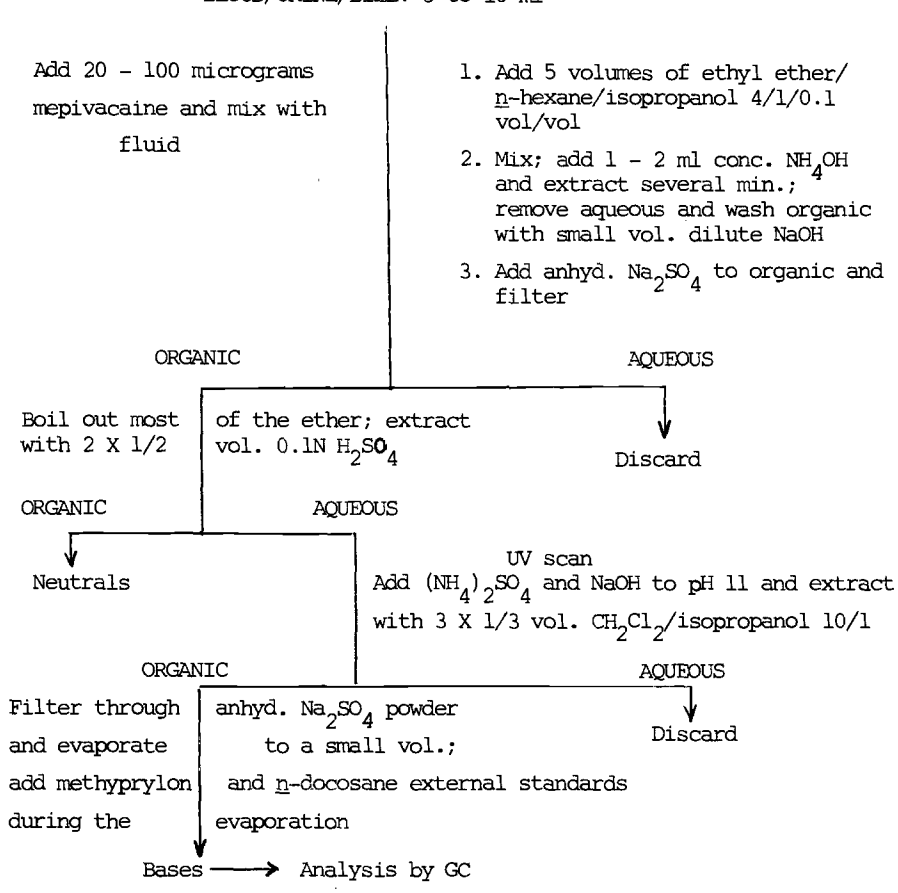

FIG. 1—*Flow chart for the extraction of meperidine and normeperidine from biological fluids.*

A given quantity of the internal standard mepivacaine was initially added to samples of body fluids to be analyzed; with tissue samples the internal standard was added during homogenization in water. Two substances were used as external standards, methyprylon and *n*-docosane, both being added to the final extraction solution. An alternate internal standard, ethoheptazine, may be used in place of mepivacaine, or the sample may be quantitated on the basis of one of the external standards. Recovery data from spiked blood, liver, and urine shown in Table 1 demonstrate that the internal standard extracts much like the compounds being analyzed, with satisfactory recoveries in all cases.

Gas Chromatography

Quantitation was accomplished by a Perkin-Elmer 900 gas chromatograph on a 2.4-m (8-ft) by 2-mm inside diameter glass column packed with 10% OV-1, 80-100 mesh. Peak areas generated by a flame ionization detector were measured. Response factors as relative areas generated for equal quantities of drug are shown in Table 2. Gas chromatographic conditions were detector, 290°C; injector, 280°C; and helium carrier gas flow, approximately 30 ml/min; the oven was held at 230°C for 4 min, then programmed at 8°C/min to 280°C. A gas chromatogram of a liver extract containing the analytes and standards is presented in Fig. 3. Qualitative GC confirmation of meperidine and normeperidine was

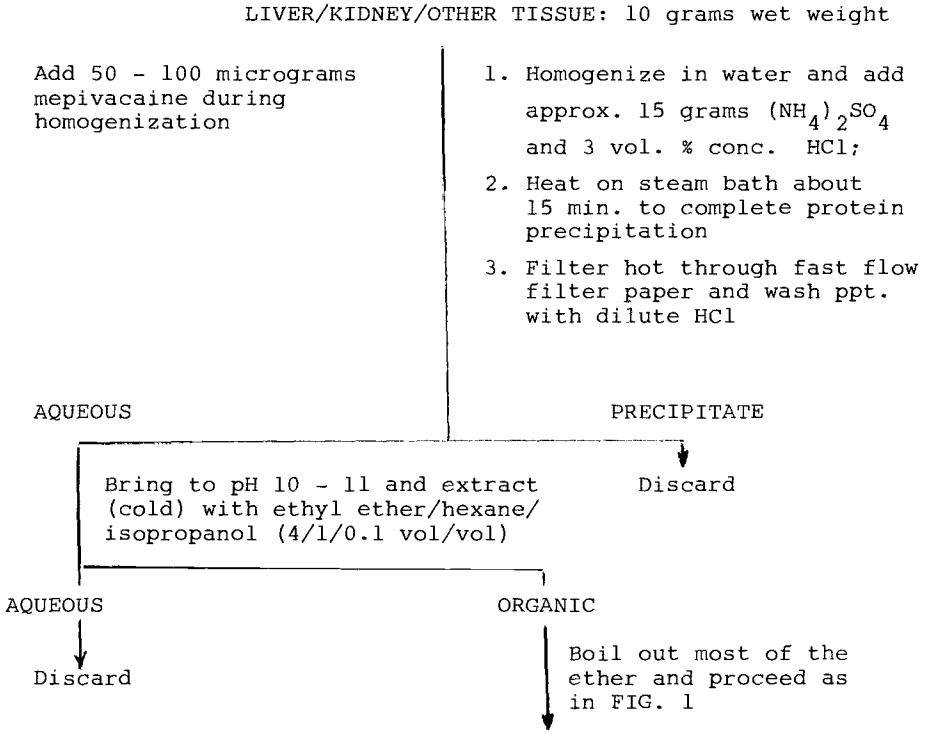

FIG. 2—*Flow chart for the extraction of meperidine and normeperidine from tissue specimens.*

TABLE 1—*Recovery of added meperidine, normeperidine, ethoheptazine, and mepivacaine from biological specimens.*

Drug	Recovered, %		
	Blood	Urine	Liver
Meperidine	94	89	80
Normeperidine	90	92	79
Mepivacaine	93	92	87
Ethoheptazine	95	90	80

accomplished on a 1.8-m (6-ft) by 2-mm inside diameter glass column packed with 3% OV-17, 100-120 mesh. In addition, a derivative of normeperidine was made with 5% ethyl chloroformate in dry chloroform by adding two or three drops of this reagent to a sample of the dry extract prior to injection. Mild heating for 10 to 20 s will cause formation of the derivative normeperidine ethylcarbamate [*9*]. Pertinent retention index [*10*] information is given in Table 3, with a number of compounds shown which elute in the vicinity of the two analytes. Ethoheptazine and diphenhydramine eluted as one symmetrical peak when these compounds were injected together on both OV-1 and OV-17. Similarly, prilocaine and ethoheptazine eluted as one peak on OV-17. A partial separation of diphenhydramine and ketamine was achieved by temperature programming on OV-17. Normeperidine and ethoheptazine were only partially resolved on the OV-17 column.

TABLE 2—*Relative response factors with flame ionization detection (FID) for compounds involved in meperidine analysis.*

Compound	Relative FID Response[a]	Involvement of Compound in the Analysis
n-Docosane	1.00	external standard
Meperidine	0.68	analyte
Mepivacaine	0.60	internal standard
Methyprylon	0.56	external standard
Ethoheptazine[b]	0.48	alternate internal standard
Normeperidine[b]	0.40	analyte

[a] Relative peak area generated by a flame detector for equal quantities of each compound injected onto a 10% OV-1 glass column.

[b] Shows peak tailing and therefore the true flame detector response may be somewhat greater if tailing is eliminated or accounted for by the integration techniques.

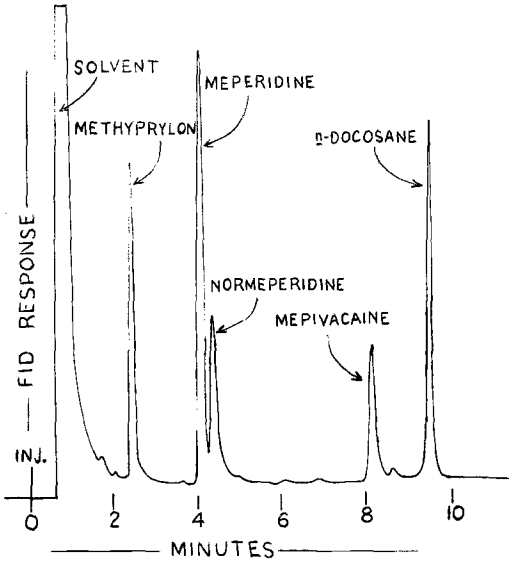

FIG. 3—*Gas chromatogram of a liver extract containing the analytes and standards.*

Qualitative Analysis

Thin-layer chromatography was used to demonstrate the presence of meperidine and normeperidine. Analtech® silica gel G soft layer plates were heated at 110 °C 1 hr or more prior to spotting samples. The R_f values in two solvent systems are shown in Table 4. System A is benzene/dioxane/diethylamine/ethanol (50:40:5:5), and System B is methanol/concentrated ammonium hydroxide (100:1.5). The mass spectra of the two analytes were obtained on a Du Pont Model 21-490B mass spectrometer; the major mass peaks are shown in Table 5. Mass spectrometer conditions were 2 s scan rate per decade to 600 mass, 70 eV, 1×10^{-7} mm mercury, and 260 °C source temperature.

TABLE 3—*Retention indexes of 25 compounds of consideration in the analysis of meperidine and normeperidine.*

Compound	Retention Index[a]		Comments
	OV-1	OV-17	
Diethylpropion	1500	1710	elutes near methyprylon
Methyprylon	1535	1863	added internal standard
Benzocaine	1570	1900	elutes near methyprylon
Methylphenidate	1745	2042	elutes near meperidine
Methoxamine	1746	2089	precedes meperidine
Meperidine	1755	2015	analyte
Normeperidine	1795	2090	analyte
Caffeine	1840	2278	commonly encountered base
Prilocaine	1851	2152	elutes after normeperidine
Benzphetamine	1860	2100	elutes near ethoheptazine
Ethoheptazine	1879	2153	alternate internal standard
Diphenhydramine	1880	2157	elutes with ethoheptazine
Antipyrine	1880	2317	with ethoheptazine on OV-1
Ketamine	1882	2230	with diphenhydramine on OV-1
Lidocaine	1903	2192	elutes after ethoheptazine
Di-*n*-butylphthalate	1934	2226	neutral contaminant
Phencyclidine	1938	2149	with di-*n*-butylphthalate on OV-1
Orphenadrine	1960	2240	common antihistamine
Methadone metabolite	2060	2377	elutes near mepivacaine
Cyclizine	2068	2348	elutes near mepivacaine
Mepivacaine	2097	2460	added internal standard
Carbinoxamine	2100	2448	elutes after mepivacaine
Homatropine	2125	2468	elutes near mepivacaine
n-Docosane	2200	2200	added external standard
Normeperidine ethylcarbamate[b]	2206	2525	normeperidine derivative

[a] See Ref *10* for an explanation of the retention index system.

[b] Will emerge from OV-1 with *n*-docosane external standard when this substance has been added. After derivatization the relative peak heights of normeperidine and *n*-docosane will change so that the derivative will be demonstrated on OV-1.

Results and Discussion

Analytical results from twelve medical examiner cases are given in Table 6. Six of these cases were certified by the medical examiner as meperidine overdoses. Case 7 was a hospital patient being treated with Demerol® who died of unexplained liver damage. Deliberate overdosing of this patient was not suspected. Cases 1, 2, and 8 had 10 mg or more of meperidine in the gastric contents; Case 6 had 2 mg and Case 11 had 4 mg of meperidine in the gastric material submitted.

The use of the mixed solvent had two advantages: (1) water was excluded from the solvent layer to a greater extent than by extracting with ether alone, and (2) ether could be boiled off prior to the back extraction, making recovery in this step nearly 100%. In extractions with aqueous acid, salts of many drugs partition into ether but not into hexane.

Widely accepted methods in analytical toxicology call for the addition of an internal standard to the specimen at the beginning of the analysis. A homologue of the drug being analyzed which differs from the analyte by a methylene unit is considered a good internal standard because of expected extraction similarities of analyte and homologue and similar GC behavior such as flame ionization detector response and proximity of elution. Ethoheptazine, although a homologue of meperidine, had two disadvantages as an internal

TABLE 4—*Thin-layer chromatography* R_f *values of compounds involved in meperidine analysis.*

Compound	Chromatography R_f Value in Solvent System	
	Benzene/Dioxane[a]	Methanol/ Ammonium Hydroxide[b]
Phencyclidine	0.95	0.52
Methylphenidate	0.90	0.74
Mepivacaine	0.90	0.78
Ketamine	0.85	0.80
Diphenhydramine	0.80	0.60
Meperidine	0.78	0.64
Nicotine	0.78	0.70
Ethoheptazine	0.76	0.40
Antipyrine	0.62	0.82
Cotinine	0.58	0.73
Normeperidine	0.43	0.28
Nicotinamide	0.27	0.70
Methoxamine	0.26	0.31

[a] A volume/volume mixture of benzene/dioxane/diethylamine/ethanol, 50:40:5:5.
[b] A volume/volume mixture of methanol/concentrated ammonium hydroxide, 100:1.5.

TABLE 5—*Major mass spectral peaks for meperidine and normeperidine by electron impact mass spectrometry.*

Meperidine		Normeperidine	
m/e Mass Peak	Relative Intensity	*m/e* Mass Peak	Relative Intensity
71	100	57	100
247	76	233	25
172	71	158	15
70	42	160	10
218	40	131	9
246	40	204	5
103	29	187	5

standard: (1) it exhibited GC peak tailing, particularly on OV-17, and (2) a number of common basic drugs had very similar retention times, as seen in Table 3. Ethoheptazine may be used if for some reason mepivacaine is not suitable. Internal standard and external standard results were found to be in agreement provided the proper response and recovery factors (see Tables 1 and 2) were used in the calculation. Generally blood and liver were analyzed with an internal standard and urine, bile, and gastric contents were analyzed with an external standard. Meperidine and normeperidine are sufficiently resolved for analysis on the OV-1 column (see Fig. 3). Although better separation occurs on OV-17, the peak tailing factor for normeperidine is more pronounced on OV-17. Table 3 calls attention to those compounds that could interfere with the analysis, particularly on a shorter column. Whether or not a particular substance will interfere depends on the ability of the column used to resolve compounds with similar retention indexes. A retention index difference of about 15 was needed for our OV-1 column in order to see two peaks with two compounds. Drugs with retention indexes below 1720 and above 1830 on

TABLE 6—*Meperidine and normeperidine concentrations in twelve medical examiner cases.*

Case	Substance Quantified	Concentration, mg/100 ml or 100 g			Remarks
		Blood	Liver	Urine or Bile	
1	meperidine	0.8	0.7	1.3, bile	suicide OD; meperidine orally
	normeperidine	1.8	6.6	1.6, bile	
2	meperidine	0.9	0.5	5, urine	suicide OD; meperidine orally[a]
	normeperidine	0.8	1.1	5, urine	
3	meperidine	2.0	1.0	...	suicide OD; meperidine orally[b]
	normeperidine	3.0	1.5	...	
4	meperidine	0.1	0.2	2.0, urine	accidental OD; meperidine by syringe
	normeperidine	0.7	1.2	6.9, urine	
5	meperidine	0.8	1.6	0.2, urine	accidental OD; meperidine by syringe[c]
	normeperidine	negative	negative	0.01, urine	
6	meperidine	0.4	0.7	2.4, urine	accidental OD; meperidine by syringe
	normeperidine	0.05	1.0	7.9, urine	
7	meperidine	...	3.0	...	undetermined; meperidine in hospital
	normeperidine	...	2.0	...	
8	meperidine	0.2	1.0	...	suicide OD with amitryptyline[d]
	normeperidine	negative	negative	...	
9	meperidine	0.4	0.5	0.9, bile	accidental drowning
	normeperidine	0.3	2.2	1.0, bile	
10	meperidine	0.4	...	1.8, urine	natural death; ruptured aortic aneurysm
	normeperidine	0.3	...	0.6, urine	
11	meperidine	0.2	...	...	natural death; acute peritonitis
	normeperidine	negative	...	...	
12	meperidine	0.2	...	...	natural death; hemorrhage from tracheostomy
	normeperidine	0.4	...	...	

[a] Codeine and pentazocine present at 0.2 mg/100 ml each in blood.

[b] Body had considerably decomposed prior to discovery and autopsy; 0.053% ethanol and 280 mg/100 ml β-phenethylamine present in the blood.

[c] Blood ethanol, 0.096% (w/v).

[d] Amitriptyline, 0.7 mg/100 ml in blood and 30 mg/100 g in liver; ethchlorvynol, 4 mg/100 ml in blood and 50 mg/100 g in the liver.

OV-1 would not interfere with this analysis. With combined OV-17 retention data, normeperidine derivative formation with ethyl chloroformate, and thin-layer chromatography on two systems, complete specificity of analysis is achieved.

The analysis of blood alone for meperidine but not normeperidine to substantiate suspected fatal overdoses seems hazardous on the basis of Table 6 and other reports dealing with meperidine blood levels. Case 4, Table 6, shows a relatively low blood meperidine concentration, yet urine concentrations and blood normeperidine concentrations show that considerable meperidine was taken. Szeto and Inturrisi [*6*] reported that a therapeutic concentration of meperidine in plasma is from 0.03 to 0.05 mg/100 ml, with the maximum normeperidine concentration being about 0.05 mg/100 ml. Liver was the only specimen available for analysis in Case 7 (Table 6); therefore the ruling of drug overdose was not made, although this seems likely. The cause of death was ascribed to an unexplained deterioration of the liver; hence it is possible that loss of liver function in this individual resulted in the accumulation of meperidine and normeperidine.

Accumulation of normeperidine in blood means meperidine was received a number of hours prior to death [*6*]. According to case history, death in Case 5 was about 30 min or less after taking meperidine intravenously. The *Registry of Human Toxicology* [*11*] re-

ports several fatal meperidine cases with blood concentrations beginning at 0.17 mg/100 ml, the highest level being 1.7 mg/100 ml. Tissue concentrations ranged from 0.3 to 16 mg/100 g, and a urine concentration of 36 mg/100 ml was reported. Normeperidine was not specified in most *Registry* cases reported through 1976. Perhaps the signal for the toxicologist to suspect a fatal meperidine overdose is when the combined meperidine-normeperidine concentration is 0.3 mg/100 ml or greater in blood. Should concentrations in other specimens be substantial, a fatal drug overdose would be confirmed.

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