TECHNICAL NOTE

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GC/MS Confirmation of Barbiturates in Blood and Urine*

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ABSTRACT: A gas chromatography-mass spectrometric method is described for the quantitative measurement of 6 commonly used barbiturates in blood and urine specimens. The targeted barbiturates are butalbital, amobarbital, pentobarbital, secobarbital, mephobarbital and phenobarbital. They are recovered along with the internal standard, tolybarb, from blood and urine using liquid extraction then alkalated to form the N-ethyl derivatives. The ethylated barbiturates have symmetrical peaks which are well separated from each other on a non-polar methylsilicone capillary column. The derivatives facilitate quantitations between 50 and 10,000 ng/mL. The day-to-day CVs for all 6 barbiturates were between 4 and 9% at 200 and 5000 ng/mL.

The method has been extended for identifying other acidic drugs and drug metabolites. They are mainly non-steroidal anti-inflammatory drugs, diuretics, and anticonvulsants. An additional 83 compounds can be qualitatively identified.

KEYWORDS: forensic science, forensic toxicology, barbiturates, gas chromatography/mass spectrometry, analysis, quantitation

Following a positive screening test it is customary to confirm the finding using an alternative analytical method. Confirmatory methods are usually chromatographic in nature such as gas chromatography and high performance liquid chromatography. For forensic applications, the most legally sound confirmatory methods employ gas chromatography/mass spectrometry (GC/MS).

Several GC/MS methods have been described for confirming the presence of barbiturates. They involve extracting then chromatographing the underivitized barbiturate (1) the methylated derivative (2–5) or the propylated derivative (6,7). Often methylation is done by flash alkylation. The residue is combined with trimethylphenylammonium hydroxide and injected into the hot injector. On column alkylation is more difficult to reproduce than precolumn alkylation occurring in the liquid phase. The excess derivatizing agent can foul the injector, the GC column and the MS source. Another approach uses extractive alkylation (5,6). The barbiturate is extracted into the organic solvent as a paired ion with a quartenary ammonium salt where it is reacted with an alkyliodide. These methods provide clean products but the reaction conditions are too mild for the method to be used to alkylate other acid drugs such as the diuretics.

Underivitized barbiturates chromatograph as tailing peaks due to their acidic property. Phenobarbital in particular tends to give a broad unsymmetrical peak (9). Adequate sensitivity is only obtained by injecting larger amounts of drug. In the author's hands unacceptable quantitative results were obtained for underivitized barbiturates at concentrations below 1 μg/mL. Barbiturate confirmation for workplace drug testing is commonly done at a cut-off near 0.3 μg/mL. Barbiturates are easily alkylated to give products with improved chromatographic properties. The methylation of mephobarbital and phenobarbital result in the same derivative (N_3-methyl-mephobarbital). Likewise methylation of metharbitol and barbital result in the same derivative (N_3-methyl-metharbital). Since the latter of each of the above pairs is the metabolite of the former, both can be present in the blood and urine after ingestion of the parent compound. For example, Hooper et al. (8) showed that serum contained 160 ng/mL mephobarbital and 170 ng/mL phenobarbital in a subject 9 days following a single 100 mg dose of mephobarbital. In the method described herein, the barbiturates are ethylated to unique products making it possible to quantitate the parent and metabolite as separate entities.

This procedure is a modification of the methods described by Kapetanovic and Kupferberg (10,11) which were used to study the pharmacokinetics of phenobarbital and p-hydroxyphenobarbital.

The blood or urine is first of all extracted with methyl-tert-butyl ether/chloroform (2:1). After evaporating, the solvent, the residue is dissolved in acetonitrile and washed with hexane to remove non-polar substances, such a lipids. Ethyl iodide and the catalyst tetramethylammonium hydroxide are added to the acetonitrile which is then heated at 70°C for 10 min. The derivitization is summarized in Fig. 1 using tolybarb, the internal standard, as the example. The reaction product is purified by adding $NaHCO_3$

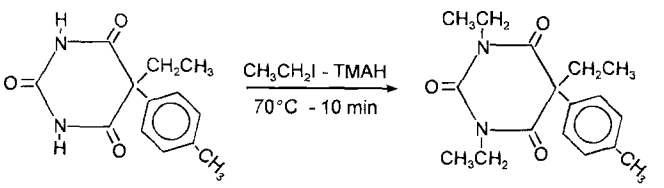

FIG. 1——*Ethylation of the internal standard tolybarb.*

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buffer to the acetonitrile then extracting the mixture with hexane/methylene chloride (3:1). The organic is reduced in volume and a portion analyzed by GC/MS.

Method

Barbiturate powders were obtained as their free acids from Health and Welfare (Ottawa) Canada. The internal standard, tolybarb (5-ethyl-5-p-tolybarbituric acid) is the para-methyl analogue of phenobarb and was obtained from Aldrich Chemical Co. (Milwaukee, WI). The non-steroidal anti-inflammatory drugs and diuretics were obtained from Sigma Chemical Co. (St. Louis, MO).

A combined methanolic stock standard was prepared to contain butalbital, amobarbital, pentobarbital, secobarbital, mephobarbital and phenobarbital, each at 1000 μg/mL. It was used to prepare both blood and urine standards at 0, 100, 200, 500, 1000, 2000, 5000, 10,000, 25,000, and 50,000 ng/mL. The 1000 ng/mL standard was used as a one point calibrator for each GC-MS run. The other standards were used in the method validation. The working internal standard was a 100 μg/mL methanolic tolybarb solution.

Ethyliodide (1-iodoethane) and tetramethylammonium hydroxide pentahydrate (TMAH) were purchased from Aldrich Chemical Co. A 25% (w/v) TMAH solution was prepared in methanol.

Sample Extraction

The following were combined in a 10 × 75 mm borosilicate test tube: 200 μL of urine or blood, 4 μL of tolybarb internal standard, 50 μL of 1 mol/L K_2HPO_4 (pH 6.8) buffer, and 1.5 mL of methyl-tert-butyl ether/chloroform (2:1) extractant. All tubes were simultaneously vortex mixed for 1 min using a Corning multitube vortex mixer. The tubes were centrifuged at 1000 × g for 2 min. The organic phase was transferred to a 5-mL glass conical tube and evaporated just to dryness under a gentle nitrogen stream at room temperature. The residue was reconstituted in 50 μL of acetonitrile. Non-polar lipids were removed by vortex mixing the acetonitrile with 200 μL of hexane for 30 s which was then aspirated to waste.

Derivatization

The barbiturates were ethylated by adding 3 μL of 25% methanolic TMAH and 10 μL of ethyliodide to the acetonitrile and heating the conical tubes for 10 min at 70°C. The derivatized barbiturates were recovered by adding 150 μL of 0.1 mol/L $NaHCO_3$ (ph 9.4) buffer and 1 mL of hexane/methylene chloride (3:1) extractant and vortex mixing the tubes for 1 min. The bottom aqueous layer was removed from the conical tube tip with a 500 μL Hamilton syringe. The tubes were centrifuged at 1000 × g for 2 min and the remainder of the aqueous layer was removed from the conical tube tip with a 50 μL Hamilton syringe. Ethyl acetate (100 μL) was added as a keeper solvent and then the organic solvent was reduced to 100 μL under a gentle nitrogen stream. A portion was transferred to an autosampler vial and 1 μL was injected into the GC/MS.

Gas Chromatography/Mass Spectrometry

A Magnum ITS40 GC/MS (Finnigan MAT, San Jose, CA) was used in the EI ionization mode. Mass spectra were collected from 55 to 500 amμ at 1 scan/s.

The gas chromatograph was initially held at 85°C for 1 min, then heated at 10°C/min to 210°C. The septum programmable injector was also initially held at 85°C but for 0.5 min, then heated at 13°C/min to 250°C. The transfer line was held at 290°C and the analyzer manifold at 220°C.

Separation was effected on a 15 m by 0.25 mm ID. by 0.25 μm film methylsilicone DB1 capillary column (J & W Scientific Inc, Folsom, CA). The capillary column was connected to a 1 m by 0.25 mm ID transfer line and to a 1 m by 0.52 mm ID retention gap using glass Presstight® connectors. Both pieces had been deactivated by the manufacturer with 5% phenylmethylsilicone. The retention gap facilitated cool on column injections using a 10 μL Hamilton syringe with a standard 2 in., 26 gage tapered needle. The helium carrier gas head pressure was 20 psi which provided a linear velocity of 45 cm/s.

Results

Total ion current chromatograms are shown in Fig. 2 for blood and urine standards containing the six targeted barbiturates at 1000 ng/mL. They are well separated from each other and have symmetrical peak shapes. In blood, tolybarb is partially separated from a later eluting endogenous peak. It has about the same peak height but does not affect the tolybarb quantitation due to the absence of the m/z = 274 amμ ion used to calculate the peak area of tolybarb. Other endogenous peaks appear in blood after tolybarb. They are presumed to be the ethyl esters of fatty acids.

In urine several endogenous substances elute just ahead of mephobarbital. They were identified as the ethyl derivatives of the caffeine metabolites 1-methylxanthine, 3-methylxanthine, 7-methylxanthine and 1,7-dimethylxanthine. Tolybarb is completely free of any interferring peaks.

Mass spectra of the ethylated barbiturates are displayed in Fig. 3. Tolybarb, phenobarbital, and mephobarbital are easily identified by their mass spectra alone.

The remaining barbiturates have mass spectra which fall into two groups. Butalbital and secobarbital both have major ions with m/z = 223 and 224 amμ. All the ions to lower mass are common to both spectra. Minor differences are present in the low intensity higher mass ions. They have different molecular weights; the protenated molecular ions having m/z = 281 and 295 amμ respectively serve to distinguish the two compounds. Talbutal and aprobarbital (not shown) have mass spectra which could be mistaken for butalbital or secobarbital. Talbutal has the same molecular weight as butalbital and both display $[M + 1]^+$ having m/z = 281 amμ. Retention time must be used to assist in making the correct identification. Aprobarbital, butalbital, talbutal, and secobarbital elute at 411, 442, 466, and 517 s, respectively.

The second group of barbiturates with similar mass spectra include amobarbital and pentobarbital. They have base peaks at m/z = 197 amμ and strong secondary ions at m/z = 212 amμ. Both have the same molecular weight and display $[M + 1]^+$ ions at 283 amμ. In addition, three other barbiturates—barbital, butobarbital, and butabarbital display the same predominant ions at m/z = 197 and 212 amu. Again retention time must be used to help distinguish these 5 barbiturates. Barbital, butobarbital, butabarbital, amobarbital and pentobarbital elute at 334, 436, 439, 468, 491 s, respectively.

In addition to the 12 barbiturates mentioned above, 9 others were investigated and found not to interfere with the method. They are metharbital, allobarbital, methohexital, hexobarbital, alphenal, heptabarbital, thiamyl, and thiopental sodium. Their retention times and major ion fragments are included in Table 1 in which data for 83 drugs are listed.

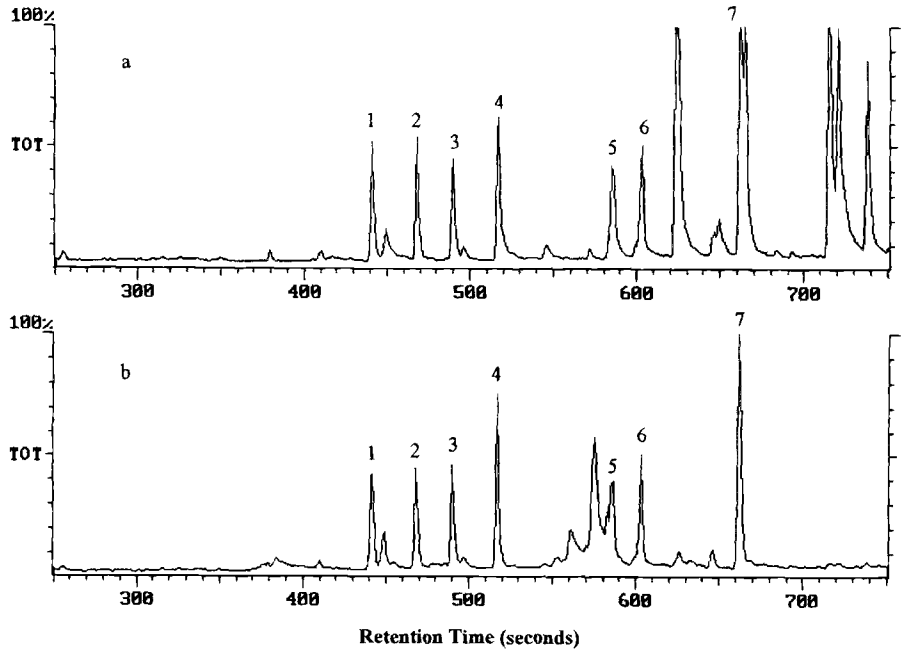

FIG. 2—*Total ion current chromatograms of barbiturate 1000 ng/mL standards in* (a) *blood and* (b) *urine: 1) butalbital, 2) amobarbital, 3) pentobarbital, 4) secobarbital, 5) mephobarbital, 6) phenobarbital, and 7) tolybarb internal standard.*

The fragmentation pathways of the respective ethylated barbiturates appear to be the same as for the underivatized drug. The actual m/z of the ions are increased by 28 or 56 depending on the number of additional ethyl groups incorporated into the ion fragment. The analogous observation (with mass increases of 14 or 28 amμ) has been made for the methyl derivatives (12).

All of the ethylated barbiturates demonstrated the protenated molecular ions in their respective mass spectra. This is probably a phenomenon associated with the ion trap. Roy (13) compared the mass spectra of 22 underivitized barbiturates obtained on an ion trap and a quadrupole mass spectrometer. An $[M + 1]^+$ ion was seen in all ion trap mass spectra. In contrast the quadrupole showed M^+ ions in the spectra of 9. Neither M^+ nor $[M + 1]^+$ appeared in the other 13 barbiturates.

A summary of the method validation data is presented in Table 2. The retention times and quantitation ions of the 6 barbiturates are included in the column headings. For the internal standard, tolybarb, they are 665 s and 274 amμ respectively. Day-to-day precision was assessed over two months at 200 and 5000 ng/mL in both blood and urine. All barbiturates had CVs between 3.9 and 8.9%.

Upper linearity was evaluated by assaying standards over the 1000 to 50,000 ng/mL range in the same run. Linearity was considered acceptable provided the assayed result was within 80 to 120% of the expected concentration. Secobarbital was linear to 10,000 ng/mL, amobarbital, pentobarbital, and mephobarbital to 25,000 ng/mL whereas butalbital and phenobarbital were still linear at 50,000 ng/mL. At concentrations where alinearity occurred, lower than expected results were always obtained for the standards. Alinearity for a given barbiturate was also heralded by the $[M + 1]^+$ ion in the mass spectrum increasing to over 40% of the base peak intensity. Their expected intensities range between 5 and 10%. Therefore the linearity appears to be limited to changes in ionization mechanisms which occur within the ion trap at higher drug concentrations.

Quantitation limits and detection limits were obtained by analyzing standards prepared by diluting the 100 ng/mL standards to 50, 20, 10, 5, 2, and 1 ng/mL. The limit of quantitation was the lowest drug concentration which gave a mass spectral fit ≥900 out of a possible 1000 a S/N ≥10 for the quantitation ion and the error in the measured concentration within ±20% of the target concentration. It was found to be 50 ng/mL for all barbiturates in blood and 20 ng/mL in urine. Similarily, the limit of detection was defined as the lowest drug concentration which gave a mass spectral fit ≥800 and a S/N ≥10. It was found to be 5 ng/mL for all barbiturates in both blood and urine.

Extraction efficiency was determined by spiking 5 blood and urine samples to 200 and 5000 ng/mL. They were extracted according to the method except that the internal standard was not added until just before the ethylation step. At the same time, 50 μL aliquots of acetonitrile containing 40 and 1000 ng of each barbiturate plus 400 ng of the internal standard were ethylated. The acetonitrile solutions represented the unextracted mass equivalent of the 200 and 5000 ng/mL standards. The extraction efficiency was calculated from the corresponding barbiturate to internal standard peak area ratios obtained in the extracted and unextracted samples. No corrections were made for solvent loss during sample preparation. These results are summarized in Table 2. Each of the barbiturates extracted equally well from urine at the two concentrations. The overall range was wider at 200 ng/mL (75–94%) than at 5000 ng/mL (91–99%). For blood, the extraction efficiencies were the same for all barbiturates at both concentrations. The overall range was 61–90%.

Carryover was assessed by injecting a zero standard after a 50,000 ng/mL urine standard. The carryover was calculated as the measured barbiturate concentration in the zero standard divided by 50,000. Phenobarbital had the highest value at 0.11%.

The extracts were found to be stable for at least 3 months when stored at room temperature in ethyl acetate. Extracts of 200, 1000, and 5000 ng/mL standards were reinjected periodically over a three month period. Each time, the analysis was calibrated with the 1000 ng/mL standard and the barbiturate concentrations calculated for the 200 and 5000 ng/mL standards. The error in the

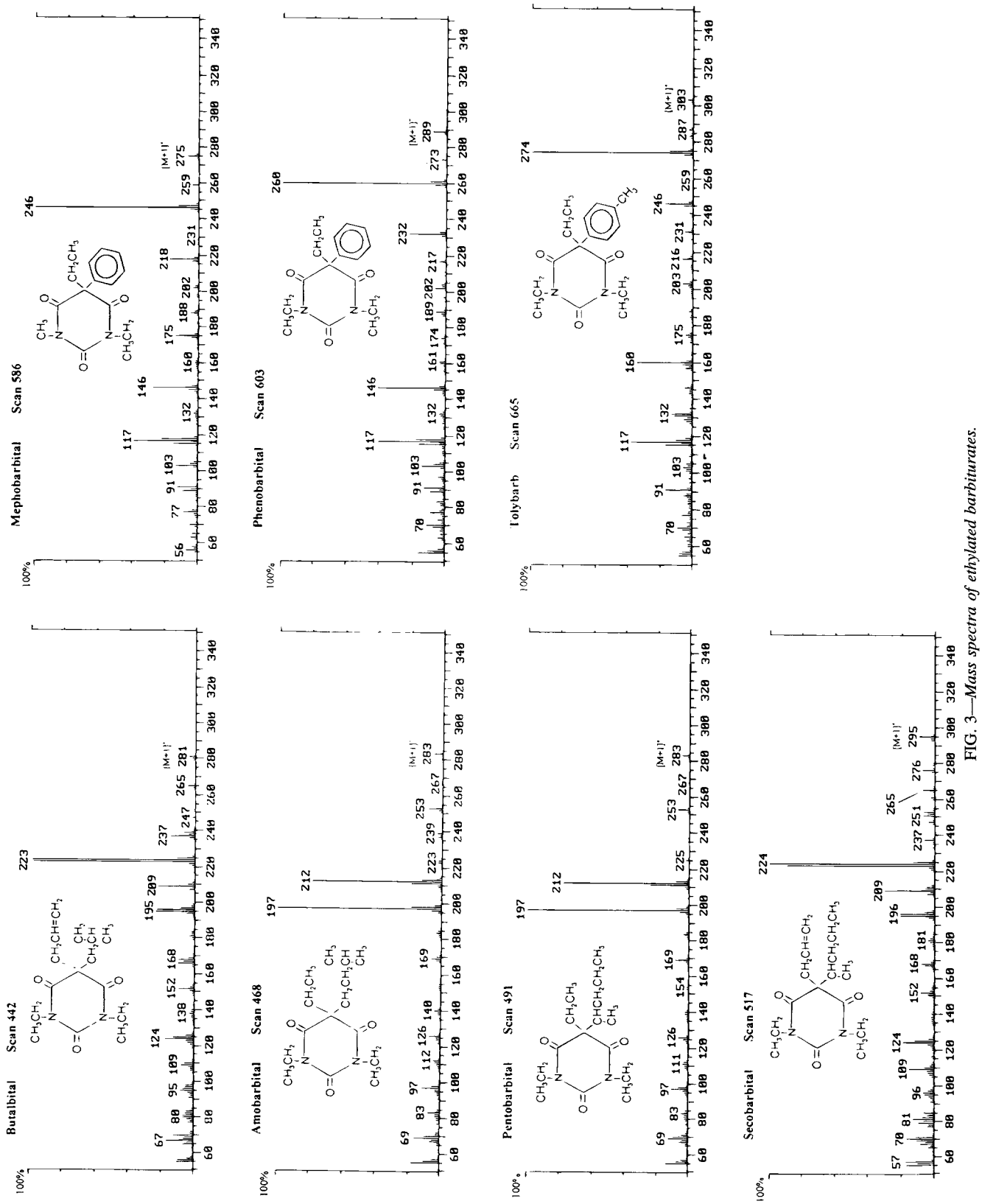

FIG. 3—*Mass spectra of ethylated barbiturates.*

TABLE 1—*Retention time and electron impact ionization of selected neutral and acid drugs.*

Ret. Time (Sec)	Drug	No. ethyl deriv **	Chemical Formula	Mol. Wt. of deriv (amu)	Mass/Charge (relative intensity)
98	paramethadione		$C_7H_{11}NO_3$	157	157(14) 129(100) 72(6) 56(20)
99	valproic acid	.	$C_{10}H_{20}O_2$	172	172(16) 129(18) 128(16) 100(100) 73(30) 57(50)
130	benzoic acid	.	$C_9H_{10}O_3$	150	150(22) 122(26) 105(100) 77(45)
146	ethosuximide	.	$C_9H_{15}NO_2$	169	170(24) 141(100 126(10) 70(33) 55(38)
180	salicylic acid (minor)	.	$C_9H_{10}O_3$	166	165(50) 121(40) 120(100) 92(75)
249	methylsalicylate	.	$C_{10}H_{12}O_3$	180	180(5) 164(26) 147(20) 133(20) 121(41) 120(100) 92(75)
249	phenylsalicylate				same mass spectrum as methylsalicylate
297	salicylic acid (major)	..	$C_{11}H_{14}O_3$	194	194(6) 178(12) 164(15) 147(45) 133(22) 121(55) 120(100) 92(55)
302	metharbital	.	$C_{11}H_{16}N_2O_3$	226	227(11) 197(62) 183(100)
334	barbital	..	$C_{12}H_{20}N_2O_3$	240	241(20) 212(65) 211(50) 197(100)
365	methyprylon		$C_{10}H_{17}NO_2$	183	184(5) 155(68) 140(100) 112(8) 98(19)
396	gentisic acid (minor)	..	$C_{11}H_{14}O_4$	210	210(36) 164(100) 136(85) 108(22) 79(15)
394	allobarbital	..	$C_{14}H_{20}N_2O_3$	264	264(18) 249(13) 235(8) 223(100) 222(60) 195(29)
401	ibuprofen	.	$C_{15}H_{22}O_2$	234	233(12) 191(10) 161(100) 119(20) 118(20) 117(23)
411	aprobarbital	..	$C_{14}H_{22}N_2O_3$	266	266(3) 248(18) 233(4) 224(63) 223(100) 209(15) 195(28)
418	methsuximide		$C_{12}H_{13}NO_3$	203	203(9) 118(100) 117(60) 103(20) 71(22) 57(45)
436	butobarbital	..	$C_{14}H_{24}N_2O_3$	268	269(5) 239(9) 223(4) 212(66) 197(100)
439	butabarbital	..	$C_{14}H_{24}N_2O_3$	268	269(4) 239(16) 212(80) 197(100)
442	**butalbital**	..	$C_{15}H_{24}N_2O_3$	280	281(4) 265(6) 237(16) 224(96) 223(100) 196(23) 209(20) 195(24)

TABLE 1—*Continued.*

Ret. Time (Sec)	Drug	No. ethyl deriv **	Chemical Formula	Mol. Wt of deriv (amu)	Mass/Charge (relative intensity)
446	N-desmethyl-methsuximide	.	$C_{13}H_{15}NO_2$	217	218(12) 118(100) 117(42) 103(14) 71(14) 57(29)
466	talbutal	..	$C_{15}H_{24}N_2O_3$	280	218(3) 262(10) 251(7) 247(5) 239(9) 238(7) 224(91) 223(100) 209(30) 195(22)
468	**amobarbital**	..	$C_{15}H_{26}N_2O_3$	282	283(6) 253(10) 239(3) 223(2) 212(82) 197(100)
480	acetaminophen	.	$C_{10}H_{13}NO_2$	179	179(66) 137(40) 109(93) 108(100) 81(20) 80(30)
491	**pentobarbital**	..	$C_{15}H_{26}N_2O_3$	282	283(4) 253(7) 225(1) 212(78) 211(23) 197(100)
494	gentisic acid (major)	...	$C_{13}H_{18}O_4$	238	238(38) 210(97) 193(10) 191(10) 164(100) 136(58)
517	**secobarbital**	..	$C_{16}H_{26}N_2O_3$	294	295(8) 276(8) 265(6) 253(6) 252(4) 251(8) 237(6) 224(100) 223(84) 209(29) 197(20) 195(20)
526	methohexital	.	$C_{19}H_{28}N_2O_3$	290	291(20) 290(14) 289(18) 275(35) 261(24) 249(100) 209(32) 178(36) 79(70)
556	theophylline	.	$C_9H_{12}N_4O_2$	208	208(100) 193(48) 180(20) 95(70)
566	hexobarbital	.	$C_{14}H_{20}N_2O_3$	264	265(5) 249(100) 183(20)
568	glutethimide	.	$C_{15}H_{19}NO_2$	245	246(7) 217(100) 188(18) 132(82) 117(70) 115(31)
576	1,7-dimethylxanthine	.	$C_9H_{12}N_4O_2$	208	208(100) 207(31) 193(18) 180(66) 179(26) 149(24) 136(99) 123(36) 81(47) 67(75)
580	7-methylxanthine	..	$C_{10}H_{14}N_4O_2$	222	222(100) 207(23) 194(40) 179(14) 166(66) 150(27) 136(58)
581	1-methylxanthine	..	$C_{10}H_{14}N_4O_2$	222	222(100) 207(24) 194(34) 179(38) 166(21) 150(82)
581	3-methylxanthine	..	$C_{10}H_{14}N_4O_2$	222	222(100) 207(32) 194(37) 179(46) 166(34) 95(60)
586	**mephobarbital**	.	$C_{15}H_{18}N_2O_3$	274	275(5) 246(100) 218(16) 175(13) 146(30) 117(38)
592	caffeine		$C_8H_{10}N_4O_2$	194	194(60) 71(56) 57(100) 56(75)
603	**phenobarbital**	..	$C_{16}H_{20}N_2O_3$	288	289(7) 260(100) 232(20) 146(40) 117(40)
612	butallylonal	..	$C_{15}H_{23}BrN_2O_3$	359	359(1) 279(53) 223(100) 195(12)
626	fenoprofen	.	$C_{17}H_{18}O_3$	270	270(58) 197(100) 91(23)
631	alphenal	..	$C_{17}H_{20}N_2O_3$	300	300(81) 285(26) 271(100) 231(32) 209(34) 158(54) 130(50) 129(59) 128(50) 104(95)

TABLE 1—*Continued.*

Ret. Time (Sec)	* Drug	No ethyl deriv **	Chemical Formula	Mol. Wt. of deriv (amu)	Mass/Charge (relative intensity)
637	flurbiprofen	.	$C_{17}H_{17}FO_2$	272	272(36) 199(100) 179(16) 178(14)
651	isobutyl-1-methyl-xanthine	.	$C_{12}H_{18}N_4O_2$	250	250(18) 194(82) 150(100) 149(74)
665	**tolybarb**	..	$C_{20}H_{28}N_2O_3$	302	303(2) 274(100) 246(15) 160(34) 117(37)
668	heptabarbital	..	$C_{17}H_{26}N_2O_3$	306	307(1) 277(100) 197(19)
681	primidone	..	$C_{16}H_{22}N_2O_2$	274	275(8) 246(45) 245(65) 146(100) 117(44)
684	naproxen	.	$C_{16}H_{28}O_3$	258	258(33) 185(100) 170(11) 153(8) 141(15) 115(10)
704	thiamyl	..	$C_{16}H_{16}N_2O_2S$	310	311(6) 281(12) 240(29) 211(34) 159(60) 109(100)
718	diflunisal*	..	$C_{17}H_{16}F_2O_3$	306	306(24) 259(35) 232(100) 204(27) 175(21)
726	indomethacin	.	$C_{21}H_{20}ClNO_4$	386	247(28) 174(100) 159(14) 131(14)
727	6-0-desmethylnaproxen	..	$C_{14}H_{16}O_3$	272	272(42) 199(100) 171(26) 141(10)
739	mefenamic acid	.	$C_{17}H_{19}NO_2$	269	269(71) 223(100) 222(28) 208(38) 194(18) 180(20)
746	ketoprofen	.	$C_{18}H_{28}O_3$	282	282(13) 238(11) 210(33) 209(66) 105(100) 77(47)
748	methaqualone		$C_{16}H_{14}N_2O$	250	250(16) 235(100)
752	acetazolamide*	...	$C_{10}H_{18}N_4O_3S$	306	307(6) 291(100) 136(20) 97(20) 72(37)
779	tolfenamic acid	.	$C_{16}H_{16}ClNO_2$	290	289(47) 243(75) 208(100) 180(32)
781	p-OH-phenobarbital	...	$C_{18}H_{24}N_2O_4$	332	332(74) 304(68) 303(100) 276(35) 275(100)
788	phenytoin	..	$C_{19}H_{20}N_2O_2$	308	308(20) 237(43) 231(28) 208(100) 165(28) 104(30) 91(30)
788	diclofenac	.	$C_{16}H_{15}Cl_2NO_2$	324	323(35) 277(14) 242(57) 214(100)
788	probenecid	.	$C_{15}H_{23}NO_4S$	313	313(3) 284(100) 213(15)
797	tiaprofenic acid* (minor)	.	$C_{16}H_{16}O_3S$	288	287(20) 215(100) 187(20) 105(20) 77(25)
800	ethacrynic acid	.	$C_{15}H_{16}Cl_2O_4$	331	330(2) 245(13) 277(64) 275(100) 247(24) 245(44) 243(64) 189(20)
833	tolmetin*	.	$C_{17}H_{19}NO_3$	285	285(23) 284(26) 270(17) 212(100) 120(21) 119(21)

TABLE 1—*Continued.*

Ret Time (Sec)	Drug	No. ethyl deriv **	Chemical Formula	Mol. Wt. of deriv (amu)	Mass/Charge (relative intensity)
838	meclofenamic acid	.	$C_{16}H_{15}Cl_2NO_2$	324	325(38) 324(12) 323(59) 277(14) 244(35) 243(42) 243(100)
842	phenylbutazone	.	$C_{21}H_{24}N_2O_2$	336	336(53) 280(33) 265(12) 183(100) 77(58)
854	ketorolac	.	$C_{17}H_{17}NO_3$	283	283(33) 238(6) 210(100) 105(95) 77(42)
861	tiaprofenic acid* (major)	..	$C_{18}H_{20}O_3S$	316	317(3) 243(100) 201(6) 165(6) 105(50)
870	zomepirac	.	$C_{17}H_{18}ClNO_3$	320	320(13) 319(30) 318(25) 248(32) 247(18) 246(100)
917	oxaprozin	.	$C_{20}H_{19}NO_3$	321	322(56) 321(92) 276(25) 249(35) 248(100) 165(30)
949	p-OH-phenylphenytoin	...	$C_{21}H_{24}N_2O_3$	352	352(10) 275(100) 252(64) 236(21) 224(18) 208(20)
958	floctafenine (minor)		$C_{20}H_{17}F_3N_2O_4$	406	347(38) 346(100) 314(54) 294(50) 287(44) 286(98) 266(25)
984	floctafenine (major)	.	$C_{22}H_{21}F_3N_2O_4$	434	361(25) 360(94) 314(69) 294(56) 287(46) 286(100) 267(27) 266(20)
996	oxphenylbutazone	..	$C_{23}H_{28}N_2O_3$	380	381(25) 380(100) 324(12) 227(92)
997	dichlorphenamide	....	$C_{14}H_{22}N_2Cl_2O_2S_2$	417	417(3) 405(16) 403(72) 401(100)
1066	hydroflumethiazide	....	$C_{16}H_{24}F_2N_3O_2S_2$	443	443(40) 428(100) 371(81) 323(41) 307(30) 266(22) 243(42)
1098	furosemide* (minor)	..	$C_{16}H_{19}ClN_2O_5S$	386	386(22) 357(22) 339(31) 96(10) 81(100)
1117	furosemide* (major)	...	$C_{18}H_{23}ClN_2O_5S$	414	414(27) 385(12) 367(12) 342(16) 96(10) 81(100)
1153	chlorthalidone	....	$C_{22}H_{27}ClN_2O_4S$	451	407(36) 406(23) 405(100) 391(16) 315(47)
1163	bumetanide	...	$C_{23}H_{32}N_2O_5S$	448	448(55) 405(41) 332(37) 313(83) 312(88) 270(48) 268(42) 240(63) 72(100)
1189	hydro-chlorothiazide	....	$C_{15}H_{24}ClN_3O_4S_2$	410	409(38) 394(37) 339(40) 337(100) 289(21) 273(28) 232(26)
1232	sulindac	.	$C_{22}H_{21}FO_3S$	384	384(25) 368(75) 248(62) 233(100)
1235	spironolactone		$C_{24}H_{32}O_4S$	416	340(80) 325(27) 267(100)
1286	bendroflumethazide*	....	$C_{23}H_{30}F_3N_3O_4S_2$	553	533(7) 442(100) 414(13)
1407	metolazone	...	$C_{22}H_{28}ClN_3O_3S$	450	436(37) 435(22) 434(100) 298(10)

*Poorly extracted from blood.

**Each bullet represents one ethyl moiety within drug derivative.

Not detected: acetaminophen, amiloride, benzthiazide, carbamazepine, carbromol, methocarbamol, chlorothiazide, chlorpropamide, cimetidine, cotinine, dyphylline, floctafenine, indapamide, mephenytoin, meprobamate, mersalyl acid, methocarbamol, nalidixic acid, nicotine, piroxicam, ranitidine, sulfamethoxazole, tenoxicam, theobromine, thiopental, triamterene, trimethorpim.

TABLE 2—*Method performance characteristics.*

	Butalbital		Amobarbital		Pentobarbital		Secobarbital		Mephobarbital		Phenobarbital	
Retention Time (s)	442		468		491		517		586		603	
Quantitation Ion (amμ)	224		212		212		224		246		260	
	Blood	Urine	Blood	Urine	Blood	Urine	Blood	Urine	Blood	Urine	Blood	Urine
Day-to-Day Precision												
200 ng/mL N	17	10	17	10	17	10	17	10	17	10	17	10
Mean (ng/mL)	202	208	199	202	198	198	191	191	194	195	199	203
CV (%)	7.9	8.7	5.0	6.4	7.0	8.9	7.2	4.7	8.6	7.3	6.3	7.0
5000 mg/mL N	16	8	16	8	16	8	16	8	16	8	16	8
Mean (ng/mL)	5014	4921	4976	4876	4872	4893	4969	4971	5099	4777	5034	4786
CV (%)	5.9	6.2	5.8	5.0	6.0	7.3	5.0	4.7	5.3	5.7	4.6	3.9
Linearity (ng/mL)	50,000		25,000		25,000		10,000		25,000		50,000	
Limit of Quantitation (ng/mL)	50	20	50	20	50	20	50	20	50	20	50	20
Limit of Detection (ng/mL)	5	5	5	5	5	5	5	5	5	5	5	5
Extraction Efficiency Range (%)												
200 ng/mL N = 5	63–82	81–85	65–82	83–94	65–82	75–83	62–85	75–84	70–84	75–84	64–84	85–92
5000 ng/mL N = 5	61–89	88–99	61–89	89–99	62–88	91–98	63–90	86–96	63–88	84–93	63–84	94–99

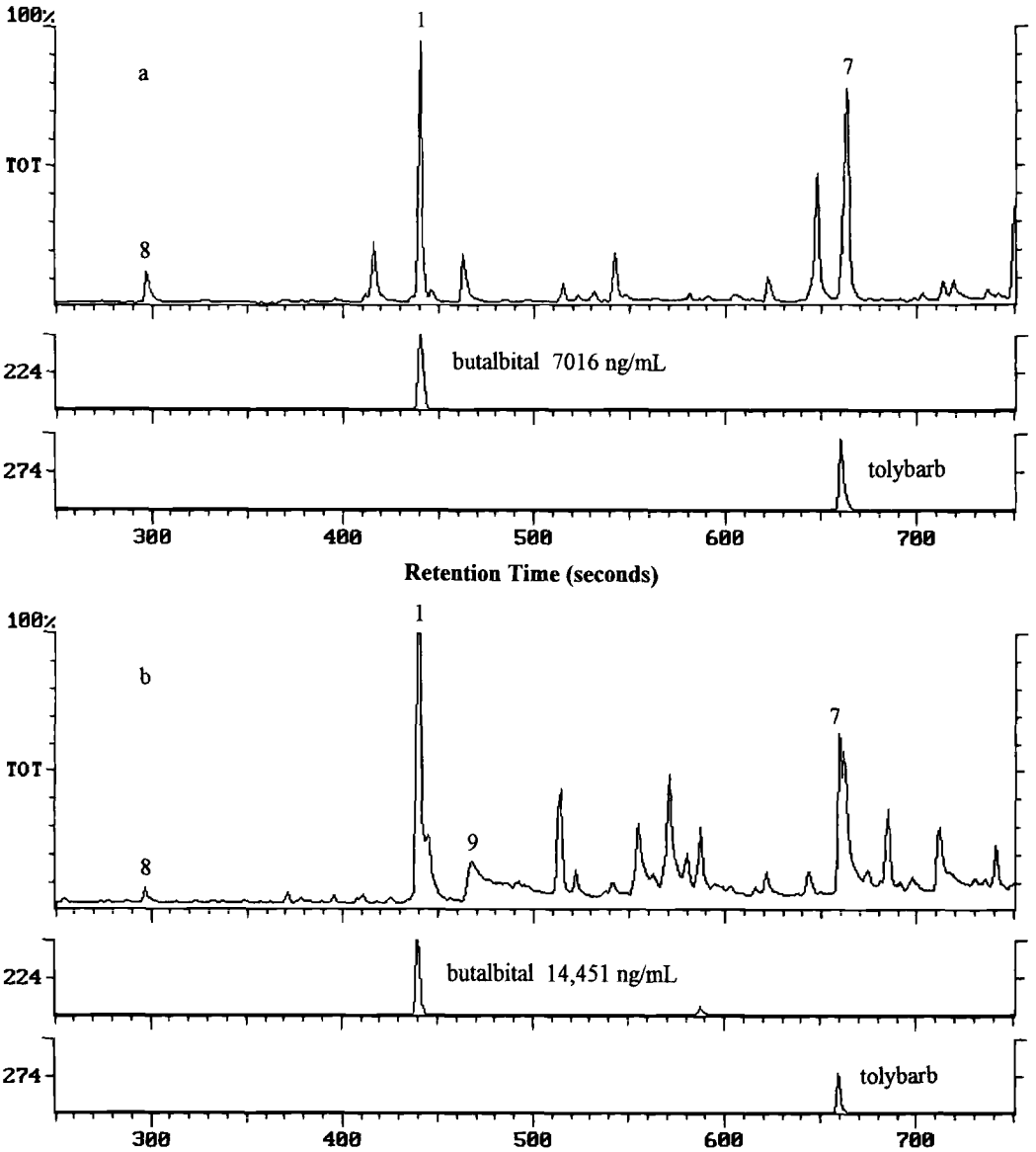

FIG. 4—*Total ion current and selected ion current chromatograms of* (a) *blood, and* (b) *urine extracts from a patient after ingesting an unknown amount of butalbital: 1) butalbital, 7) tolybarb internal standard, 8) salicylic acid, and 9) acetaminophen.*

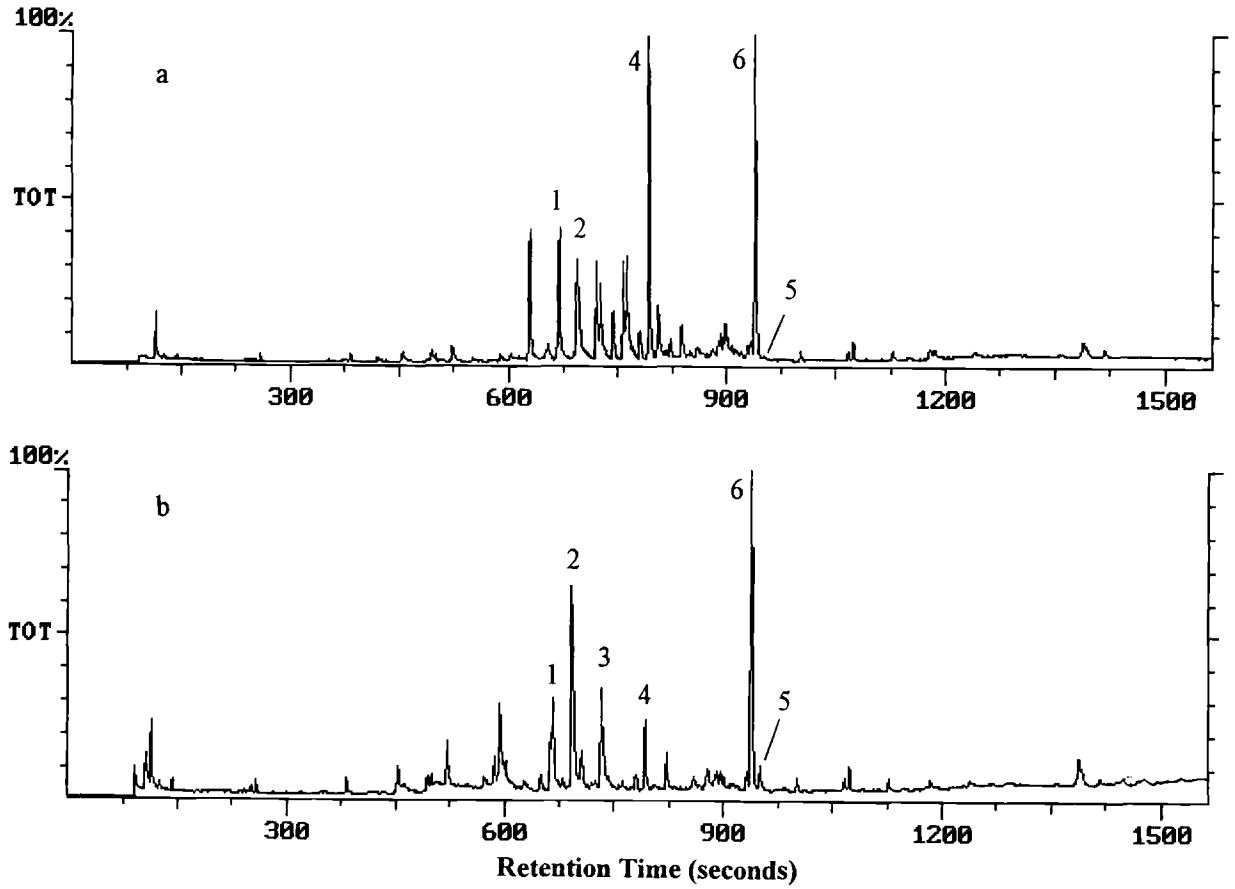

FIG. 5—*Total ion current chromatograms from* (a) *blood and* (b) *urine samples from a patient taking therapeutic doses of phenytoin and naproxen: 1) tolybarb, 2) naproxen, 3) 6-O-desmethylnaproxen, 4) phenytoin, 5) p-OH-phenytoin, and 6) a phthalate.*

calculated concentration was always less than ±15% of the target concentration for all 6 barbiturates. In separate experiments, it was discovered that extracts which had dried to residue could not be used to report quantitative results.

Discussion

All of the barbiturates behaved identically throughout the method, facilitating the use of a single internal standard. The method performs equally well on blood or urine specimens. The extractant, methyl-tert-butyl-ether/chloroform (2:1) is polar enough to recover the barbiturates but does not cause aggregation of whole blood as does chloroform alone.

As soon as the extractant had evaporated, the residue was removed from the nitrogen stream to prevent sample loss. Drying the derivatized extract was found to be more critical than the underivatized. Joern (6) illustrated the volatile nature of the early eluting propylated barbiturates. He recommended taking the derivatized extracts just to dryness, then reconstituting them in the injection solvent. Here, the problem was solved by adding ethyl acetate as a keeper solvent to the hexane/methylene chloride (3:1) after the extraction. The organic was reduced to 100 μL then 1 μL injected.

Figure 4 displays chromatograms of blood and urine extracts of a patient presenting to the emergency department shortly after ingesting an unknown quantity of Fiorinal tablets which contain butalbital, acetylsalicylic acid, and caffeine. The butalbital was 7016 ng/mL in the blood and 14,451 ng/mL in the urine. Salicylate was detected in both blood and urine as its diethyl derivative. The concentrations were not determined. Acetaminophen was also detected as its ethyl derivative in the urine sample but not in the blood. Two hours after ingesting a single 100 mg dose of butalbital, the average peak blood concentration from 5 subjects was 2140 ng/mL (14). The mean elimination half-life was 61 h. From this data, it is likely that the patient in Fig. 4 ingested 400 to 600 mg of butalbital.

Ethylation of barbiturates is complete by 10, 5, and 3 min at 37, 56, and 70°C. Once formed, the ethylated barbiturates are not decomposed by heat or the alkaline TMAH catalyst. Propylation, in contrast, occurs too slowly at 70°C resulting in partial decomposition before the derivative has had time to form and hence produces lower yields. Propylation proceeds satisfactorily at 37°C. However other drugs of interest, the non-steroidal anti-inflammatory drugs (NSAID) and the diuretics, gave poor yields at lower temperatures. Satisfactory yields of all 3 classes of drugs were obtained when ethylation was conducted at 70°C for 10 min.

Table 1 summarizes the retention times and mass spectral data of 83 acidic compounds that were subjected to the procedure. Each pure compound was added to acetonitrile, one at a time, and alkylated according to the procedure. The oven and injector programs of the gas chromatograph were extended to heat to 285°C in order to elute the higher boiling compounds. In most cases only one peak appeared in the chromatogram and the mass spectrum of the expected derivative contained the M^+ or $[M + 1]^+$ ion. Each of the drugs were then spiked into blood and urine at 5000 ng/mL and subjected to the entire procedure to ensure they could be adequately extracted. Qualitatively, all drugs extracted well from

urine spikes. Diflunisal, acetazolamide, tiaprofenic acid, tolmetin, furosemide, and bendroflumethazide were poorly extracted from blood spikes.

The blood and urine from a patient taking therapeutic amounts of naproxen and phenytoin were assayed and the chromatograms are shown in Fig. 5. The blood sample showed predominantly phenytoin and naproxen parent compounds plus a small amount of p-OH-phenytoin. Numerous other peaks in the 700 to 900 second region are often present in blood and serum extracts. They are probably the ethyl esters of free fatty acids. The urine extract is void of these interferences. In addition to phenytoin, p-OH-phenytoin and naproxen, the primary urinary naproxen metabolite, 6-O-tdesmethylnaproxen, was also identified.

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