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Drug Concentration in Selected Skeletal Muscles

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ABSTRACT: We evaluated the homogeneity of drug concentrations in muscle in 14 cadavers, comprising 11 drug overdoses and three cases of chronic therapeutic drug use. Analyses were performed on samples from twelve named muscles and femoral venous blood. Standard analytical techniques and instrumentation were used throughout. There was marked within-case variability in drug concentrations with highest:lowest concentrations ranging up to 21.7. Overall highest concentrations were found in the diaphragm and mean diaphragm:blood ratios ranged from 1.1 (temazepam, two cases) and 1.2/1.3 (paracetamol, six cases) up to 6.5/13.5 (amitriptyline, three cases) and 5.3/21.3 (propoxyphene, four cases). Excluding the diaphragm, mean muscle:blood ratios ranged from 0.4 (prothiaden), 0.5 (temazepam), and 0.7 (paracetamol) up to 3.7 (temazepam), 4.3 (propoxyphene) and 5.7 (amitriptyline). We suggest that muscle is suitable for qualitative analysis but not for quantitative corroboration of a blood sample or as a quantitative alternative to blood.

KEYWORDS: forensic science, forensic toxicology, postmortem redistribution, muscle, temazepam, paracetamol, prothiadin, thioridazine, amitriptyline, propoxyphene

An increasing awareness of the problem of postmortem changes in drug levels in blood (1–4) has prompted a search for corroborative or alternative biological samples. Skeletal muscle has been suggested as a suitable candidate (5) but one previous study (6) indicated that there might be variability in drug concentration between muscle samples. Multiple random sampling of leg muscle in a limited number of cases has shown that within-case variability of drug concentrations may be extreme (7). We set out to explore this problem in greater detail through standardized multiple sampling of named muscles in case fatalities.

Methods

Suspected cases of drug poisoning and documented chronic therapeutic drug usage were identified before autopsy. The details of the fourteen selected cases are summarized in Table 1. The autopsy protocol allowed for sampling of twelve muscles and femoral blood. Before obtaining a femoral vein blood sample by needle and syringe the vessel was cross clamped proximally. Muscle samples were obtained from the midparts of the pectoralis major, sternomastoid, deltoid, biceps, triceps, brachioradialis, rectus abdominis, sartorius, vastus lateralis, gastrocnemius, gluteus maximus and the diaphragm (right dome laterally).

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All samples were stored at −20°C until analysis. From the tissue samples, 5.0 g were accurately weighed. The sample was finely chopped with scissors and homogenized in 15 mL distilled water using a blender (Ultra Turrax T25, Janke and Kunkel, IKA Laboritechnik) for 1 min at 8500 rpm, and then subsequently at 9500, 13,500, 20,500 and 24,000 rpm for 1 min each. Quantities of 1 mL femoral blood and 1 g homogenate were used for extraction. All analyses were performed in duplicate to within 5% of the mean value. The assays employed follow the standard investigative procedures used and developed within the laboratory to determine drug concentrations in various body fluids and tissues. Quantitation was performed using a 6 point calibration curve for each drug, with a minimum acceptable correlation coefficient of 0.99.

For benzodiazepine analysis, internal standard solution (prazepam, 10 μL, 1 mg/mL) and phosphate buffer (pH7.4, 1 mL) was added to the extraction sample and mixed briefly on a vortex mixer. Diethyl ether (4 mL) was added and the sample rotated for 15 min (Spiramix 10, Denley, UK). The organic layer was aspirated and diethyl ether (4 mL) was added to the extraction sample and mixed again. The combined organic layer was evaporated to dryness at 50°C under a stream of dry air. The extracts were further purified by partition between acetonitrile (1 mL) and heptane (2 mL). The acetonitrile layer was aspirated and evaporated to dryness. The residue was resuspended in methanol (100 μL) and a sample (20 μL) was injected into the HPLC system. The HPLC conditions were as follows: instrument Gilson isocratic LC pump 307 with Gilson ultraviolet spectrophotometric detector 118 (wavelength 240 nm) and Waters autoinjector 717+; column, Apex II ODS 5 μm, 150 by 4.6 mm with guard column 20 mm; mobile phase, phosphoric acid (180 mL, 10 mM), disodium hydrogen phosphate (20 mL, 10 mM), acetonitrile (100 mL) and methanol (100 mL); flow rate 1.0 mL/min. Under these conditions the retention times for temazepam and prazepam (i.s.) were 5.1 and 15.4 min respectively. The calibration showed linearity between 0–20 μg/mL, $r = 0.9991$).

For paracetamol analysis, extraction was performed by the same procedure as for benzodiazepines. 2-acetoamidophenol (100 μL, 200 μg/mL, or 100 μL, 2 mg/mL for coproxamol cases) was used as the internal standard. The extracted residue was resuspended in methanol (1 mL) and a sample (20 μL) was injected into the HPLC system. The HPLC conditions were as follows: wavelength 255 nm; mobile phase, acetonitrile (100 mL), acetic acid (50 mL), diluted to 1000 mL with distilled water. Under these conditions, the retention times for paracetamol and 2-acetamidophenol (i.s.) were 3.3 and 5.8 min. The calibration showed linearity between 0–200 μg/mL, $r = 0.9980$ and between 0–615 μg/mL, $r = 0.999$ for the coproxamol cases.

For prothiadin, amitriptyline and thioridazine analysis, doxepin (100 μL, 50 μg/mL) was used as internal standard. For fluoxetine and thioridazine analysis (case 7) amitriptyline (10 μL, 100 μg/mL)

TABLE 1—*Case data.*

Cases	Age/Sex	Height/Weight (cms/kg)	History/Autopsy Notes	Postmortem Interval (h)†	Refrigeration (h)	Body Position‡
1*	48M	168/75	Alcoholic/Manic depressive; pulmonary thromboembolism	20.5	20.5	supine
2*	38F	161/86	Acute alcohol poisoning	16.5	15.5	supine
3	72F	155/46	Severe cor pulmonale	42.5	41.5	supine
4	69M	185/85	Theophylline, paracetamol overdose; died in hospital	17.5	17.5	supine
5	60F	153/81	Overdose, died in hospital	10	10	supine
6	50F	143/65	Reactive depression	66.5	65	supine
7*	28F	164/72	Chronic alcoholism	70	0.5	prone
8	32M	167/68	Reactive depression, died in ambulance	16.5	16.5	supine
9	60M	157/49	Severe depression for 10 years	99	97.5	supine
10	34F	162/uk	Alcoholic, reactive depression	16	12	supine
11	58M	176/84	Arthritis, reactive depression	86	85	supine
12	35M	157/52	Endogenous depression	21.5	20	supine
13	37M	166/68	Depression/Schizophrenia	57.5	32	prone
14	30M	172/80	Paranoid schizophrenia	71	70	supine

uk-Unknown.
*Chronic therapeutic drug use.
†Best estimate.
‡Prior to refrigeration.

was used as internal standard. To the extraction sample containing internal standard, sodium hydroxide (2 mL, 0.5 N) was added.

Heptane:isoamyl alcohol (98.5:1.5), 4 mL was added and mixed for 15 min. The organic layer was aspirated and a further 4 mL heptane:isoamyl alcohol was added to the extraction sample. The organic layers were combined and back extracted with sulfuric acid (2 mL, 0.1 N). The acid layer was made alkaline with carbonate: bicarbonate buffer (1 mL, pH9.0) and re-extracted in toluene:isoamyl alcohol (85:15, 3 mL) for 15 min. The organic layer was aspirated and evaporated to dryness at 50°C under a stream of dry air.

For prothiadin, amitriptyline and thioridazine, the residue was resuspended in methanol (100 μL) and a sample (20 μL) was injected into the HPLC system. The HPLC conditions were as follows: wavelength 255 nm; mobile phase, phosphate buffer (300 mL, pH 3.0), n-nonylamine (600 μL), acetonitrile (200 mL). Under these conditions retentions times for prothiadin, amitriptyline, thioridazine and doxepin (i.s) were 5.3 min, 5.2 min, 11.3 min, and 3.3 min respectively. The calibration showed linearity between 0–25 μg/mL for prothiadin, $r = 0.990$, amitriptyline, $r = 0.9971$ and 0–10 μg/mL for thioridazine, $r = 0.990$).

For fluoxetine/thioridazine, the residue was resuspended in ethyl acetate (20 μL) and a sample (2 μL) was injected into a gas chromatograph connected to a mass spectrophotometer. The GC-MS conditions were as follows: instrument, Fisons GC 8000 series equipped with quadropole mass analyser MD800; column, CPSil 5 CB-MS, 30 m by 0.25 mm ID with a 0.25 μm film thickness; temperature program, initial temperature 100°C (1 min hold), ramped to 300°C at 20°C/min (3 min hold); injection port temperature 250°C; carrier gas, Helium (linear velocity 35 cm/s); ionization energy 70 eV; transfer line temperature 250°C. Under these conditions, the retention times for amitriptyline (i.s), fluoxetine and thioridazine were 11.7 min, 9.6 min, and 20.6 min respectively. The calibration showed linearity between 0–4 μg/mL for fluoxetine, $r = 0.9955$ and thioridazine, $r = 0.9904$.

Peaks corresponding to drugs of interest were identified by a combination of their full mass spectra and retention times. Quantitation of both sample and standard peaks was performed using the total ion current (TIC). The peak area ratio was normalized to the internal standard and the sample drug concentration calculated from the relevant calibration curve.

For dextropropoxyphene analysis, 100 μL dothiepin (50 μg/mL) was used as internal standard. Extraction was as described for prothiadin. The residue was resuspended in 50 μL toluene:isoamyl alcohol (85:15) and a sample (1 μL) was injected into a gas chromatograph connected to a mass spectrophotometer. The GC-MS conditions were as follows: instrument, Fisons GC 8000 series equipped with quadropole mass analyser MD800; column, CPSil 5 CB-MS, 30 m by 0.25 mm ID with a 0.25 μm film thickness; temperature program, initial temperature 100°C (1 min hold), ramped to 300°C at 10°C/min (5 min hold); injection port temperature 220°C; carrier gas, Helium (linear velocity 35 cm/s); ionization energy 70 eV; transfer line temperature 250°C. Under these conditions, the retention times for dextropropoxyphene and dothiepin were 11.3 min and 12.4 min respectively.

The retention times of dextropropoxyphene and dothiepin (i.s.) were identified by their full mass spectra at 11.3 min and 12.4 min respectively. Quantitation of both sample and standard peaks was performed using m/z 58 when scanning the sample in selective ion monitoring mode (SIR). The ions monitored were m/z 58.07, 117.09 and 193.10 for dextropropoxyphene and m/z 58.07, 202.04 and 295.14 for dothiepin. The peak area ratio was normalized to the internal standard and the sample drug concentration calculated from the calibration curve. The calibration curve showed linearity between 0–10 μg/mL, $r = 0.9933$.

Results

Of the 14 cases, 11 were suicidal poisonings, of which four (cases 11–14) poisonings were due to Coproxamol (British Approved Name; paracetamol 325 mg and propoxyphene 32.5 mg). The remaining three (cases 1, 2 and 7) were cases of documented chronic therapeutic drug administration. The relevant case data is summarized in Table 1. The analytical results together with tissue

TABLE 2—*Concentration (μg/mL or μg/g) of temazepam in femoral vein blood and skeletal muscle samples (cases 1, 2, 3).*

Sample	Case 1* pH	Case 1* Temazepam	Case 2* pH	Case 2* Temazepam	Case 3 pH	Case 3 Temazepam
Femoral vein		0.1		0.8		4.3
Pectoralis major	5.7	0.5	6.0	0.4	6.2	2.0
Sternomastoid	6.0	0.6	6.0	0.8	6.6	2.1
Deltoid	6.0	0.5	6.0	0.8	6.5	2.4
Biceps	6.0	0.5	5.9	0.8	6.1	2.0
Triceps	6.0	0.5	6.0	0.8	6.1	2.0
Brachio-radialis	6.0	0.5	6.0	0.7	6.2	2.1
Rectus abdominis	6.0	0.5	6.0	0.8	6.7	2.3
Sartorius	6.1	0.5	5.9	0.7	6.5	2.2
Vastus lateralis	6.2	0.5	6.0	0.9	6.2	2.4
Gastrocnemius	6.1	0.5	6.0	0.8	6.2	2.2
Gluteus maximus	6.1	ns	5.9	0.7	6.9	2.6
Diaphragm	6.0	0.6	5.8	0.8	6.7	4.7

ns = No sample.
*Chronic therapeutic drug use.

pH are summarized in Tables 2–6. Summary results are shown in Table 7.

Seven drugs with a broad range of volume of distribution (Vd) in cases with a wide range of postmortem interval and refrigeration (Tables 1 and 7) are included in this study. As with all case material studies there are many uncontrolled variables prior to sampling. There is no obvious relationship between postmortem interval, postmortem refrigeration time and body position with the pattern of analytical results. Drug concentrations in the diaphragm are almost invariably higher than in other muscles but the difference is less marked in the cases of chronic therapeutic drug usage (cases 1, 2 and 7) when contrasted with the others. The variability of drug concentrations in muscle, excluding diaphragm, is generally greater for those drugs with a high Vd (Table 7) but it is not always true that drugs with a low Vd show little variability. Similarly the variability of drug concentration in muscle is not clearly a feature of acute overdose as contrasted with chronic therapeutic use (Table 7). There is no consistent pattern of hierarchy of muscles in terms of drug concentration, with the exception of the diaphragm. Even the mean muscle drug concentration varies considerably in its ratio to the femoral venous blood concentration, ranging from 0.4–5.7. Relatively high drug concentrations are seen in the rectus abdominis muscle in two cases with acute drug overdosage and a prolonged postmortem interval (cases 9 and 11) as well as one case with a short postmortem interval (case 10), but this must be viewed against the general variability in the pattern and, in the absence of data on gastric residue analysis, merely raises the possibility of gastric residue diffusion.

TABLE 3—*Concentration (μg/mL or μg/g) of paracetamol and prothiadin in femoral vein blood and skeletal muscle samples (cases 4, 5, 6).*

Sample	Case 4 pH	Case 4 Para	Case 5 pH	Case 5 Para	Case 5 Prothiadin	Case 6 pH	Case 6 Prothiadin
Femoral vein		87		288	7.9		6.0
Pectoralis major	5.8	195	6.6	122	1.2	6.2	17.8
Sternomastoid	5.8	214	7.2	245	0.4	6.1	18.2
Deltoid	5.9	184	6.4	176	5.4	6.0	18.5
Biceps	5.9	198	7.1	150	5.1	6.0	15.7
Triceps	5.9	211	6.0	168	4.0	5.8	14.8
Brachio-radialis	6.0	225	7.1	163	2.0	6.1	16.3
Rectus abdominis	6.0	206	7.2	209	2.5	6.3	16.6
Sartorius	6.0	250	6.2	222	6.0	6.1	17.6
Vastus lateralis	6.0	187	6.1	256	2.6	6.0	18.5
Gastrocnemius	6.1	197	6.8	261	2.2	6.0	16.8
Gluteus maximus	6.1	163	7.0	212	3.2	5.9	17.7
Diaphragm	6.0	300	6.7	341	11.8	6.8	51.9

Para = paracetamol.

Discussion

The earliest extensive study of drug levels in skeletal muscle was undertaken by Danish colleagues (6) but interest remained dormant until recently (5,7). As a postmortem sample for toxicological analysis, skeletal muscle has the advantage that it is present in large quantities and is affected by decomposition later than the viscera. In the light of what is known of diffusion artefacts influencing drug levels in blood (1–4), muscle has the distinct advantage that samples can be obtained from peripheral sites, well away from drug reservoirs in gastric residue and viscera such as liver and lung. It is generally agreed (5–7) that skeletal muscle is a useful biological sample for qualitative toxicological analysis. Its use for quantitative analysis has been endorsed by some (6), advanced with reservations (5) and disputed (7).

The earliest study (6) was made prior to a general awareness of postmortem diffusion artefacts but offers useful data because named muscles were sampled (biceps and quadriceps) and compared with a peripheral blood sample in a large number of cases. Of the two more recent studies, the one (5) utilized thigh muscle (not otherwise defined) and aortic blood, which is known to be susceptible to both drug redistribution from lung (8,9) as well as gastro-oesophageal residue diffusion (10,11). The other recent study (7) randomly sampled leg muscle (not otherwise specified) and made comparison with a femoral vein sample but in a limited number of cases. The present study utilizing 12 anatomically defined muscle samples and femoral venous blood offers detailed information in a limited number of cases. All four studies offer complementary information which taken together indicates that there is considerable within-case variability in the drug concentration in skeletal muscle with no apparent pattern overall. This inevitably compromises quantitative interpretation based upon drug levels in muscle or muscle:blood ratios.

It has been suggested that the muscle:blood ratio might serve as an indicator of the time lapse between drug ingestion and death with short survival times associated with low ratios and long survival times associated with high ratios (5). Data from the four cases of propoxyphene poisoning in this study (cases 11–14) suggest otherwise since propoxyphene overdose generally kills within one hour or less but muscle:blood ratios are high in all four instances. It seems likely that the Vd of a drug as well as the time lapse between ingestion and death is a major factor influencing the muscle:blood ratio (7). In the present study drugs with a low volume of distribution (temazepam; 0.8–1.0 L/kg and paracetamol; 0.8–1.0 L/kg) tended to exhibit less variation between sites, as reflected in the lower c.v. values, than those drugs with a higher volume of distribution.

The observation in this study that drug concentrations in the diaphragm are typically far higher than other muscles corroborates the observation of Christensen (6). In most cases the diaphragm: femoral ratio was greater than the muscle:femoral ratio, a pattern which appeared to be accentuated by a high Vd. It seems likely that this is the result of a greater blood flow to the diaphragm, a muscle of respiration, than to other skeletal muscle, so that equili-

TABLE 4—*Concentrations (μg/mL or μg/g) of fluoxetine, thioridazine and amitriptyline in femoral vein blood and skeletal muscle (cases 7, 8).*

Sample	Case 7* pH	Fluoxetine	Thioridazine	Case 8 pH	Thioridazine	Amitriptyline
Femoral vein		0.3	0.2		0.4	1.9
Pectoralis major	6.9	0.5	0.5	6.2	0.6	5.8
Sternomastoid	6.7	0.03	0.4	6.6	0.8	2.5
Deltoid	7.1	nd	0.3	6.2	1.2	5.4
Biceps	7.0	nd	0.7	6.2	0.9	3.5
Triceps	7.2	0.4	0.5	6.6	0.9	1.8
Brachio-radialis	7.3	0.4	0.6	6.2	1.4	3.1
Rectus abdominis	6.6	0.6	0.7	6.3	0.5	3.8
Sartorius	7.1	0.2	1.1	6.0	1.1	5.7
Vastus lateralis	7.2	0.2	0.4	6.3	0.4	3.8
Gastrocnemius	6.9	0.4	0.5	6.3	1.3	3.1
Gluteus maximus	7.0	0.3	0.4	6.1	0.5	8.3
Diaphragm	6.5	1.1	1.3	6.4	2.3	12.7

nd = not detected.
*Chronic therapeutic drug use.

TABLE 5—*Concentration (μg/mL or μg/g) of amitriptyline in femoral vein blood and skeletal muscle samples (cases 9, 10).*

Sample	Case 9 pH	Amitriptyline	Case 10 pH	Amitriptyline
Femoral vein		13.1		1.8
Pectoralis major	6.4	17.2	6.2	10.9
Sternomastoid	6.6	31.2	6.3	9.3
Deltoid	6.3	16.4	6.1	11.0
Biceps	6.2	16.4	6.1	10.5
Triceps	5.9	9.2	6.2	9.6
Brachio-radialis	6.1	14.0	6.1	10.0
Rectus abdominis	6.7	21.0	6.3	13.1
Sartorius	6.3	15.4	6.1	10.8
Vastus lateralis	6.5	8.4	6.2	8.7
Gastrocnemius	5.8	8.9	6.2	9.1
Gluteus maximus	6.2	4.9	6.2	10.7
Diaphragm	6.4	85.0	6.2	24.4

bration between blood and diaphragm occurs more rapidly. It may also be that drug levels in the diaphragm are artefactually elevated postmortem as a result of diffusion from reservoirs of high concentration in the liver and lung (1–4) or from gastric residue (10,11). If the explanation for the high drug levels found in the diaphragm is indeed a greater blood flow then variability in blood flow to individual skeletal muscles, consequent on varying physical activity and posture prior to death, may account for the variability in drug concentrations between different skeletal muscles. Another potential confounding factor not explored in this study, is the possibility of between-muscle variability in the rate of conversion of parent drug to metabolites.

Variability of drug concentrations between skeletal muscles has implications for the calculation of total body drug load by the method of "tissue additions" (12,13). This method simply involves measuring the drug concentration in tissues having a major drug load as a consequence of bulk (skeletal muscle and fat) or as a result of preferential drug concentration (liver and lung), multiplying the concentration by tissue mass and adding these tissue loads. Adding gastric drug residue gives the minimum ingested dose. Such a calculation

TABLE 6—*Concentrations (μg/mL or μg/g) of paracetamol and dextropropoxyphene in femoral vein blood and skeletal muscle (cases 11, 12, 13, 14).*

Sample	Case 11 pH	Para	DPX	Case 12 pH	Para	DPX	Case 13 pH	Para	DPX	Case 14 pH	Para	DPX
Femoral vein		227	0.9		179	1.0		193	2.5		63	1.1
Pectoralis major	6.0	279	3.1	5.7	188	3.0	6.1	274	5.0	6.3	103	3.7
Sternomastoid	6.0	325	5.5	5.8	188	3.3	6.0	240	4.3	6.5	110	2.8
Deltoid	6.0	294	3.6	5.9	176	2.8	6.0	259	5.3	6.4	100	3.0
Biceps	6.0	258	3.3	6.0	194	2.7	6.1	256	5.5	6.2	104	3.3
Triceps	5.9	211	2.0	6.0	206	3.0	6.1	249	4.8	6.1	104	3.3
Brachio radialis	5.9	306	4.2	5.9	189	2.7	6.1	259	5.3	6.1	103	2.7
Rectus abdominis	6.1	415	6.4	5.7	211	3.7	6.1	225	4.3	6.1	110	3.8
Sartorius	5.8	256	3.4	6.0	198	2.8	5.9	232	5.8	6.2	88	3.1
Vastus lateralis	5.9	201	2.6	6.0	206	2.8	6.0	235	5.3	6.1	101	1.9
Gastrocnemius	6.0	171	2.2	6.1	198	3.2	6.0	252	7.9	6.1	92	2.5
Gluteus maximus	5.9	285	7.3	6.0	191	2.9	6.0	240	6.1	6.1	92	
Diaphragm	6.2	542	19.8	6.0	237	9.1	6.1	253	14.4	6.2	140	6.0

Para = paracetamol.
DPX = dextropropoxyphene.

TABLE 7—*Summary of analytical results.*

Case	Drug	Vd (L/kg)	Femoral Blood Concentration (mg/L)	Diaphragm Concentration (mg/kg)	Mean Diaphragm: femoral ratio	Mean Muscle Concentration (mg/kg)*	Range	Ratio of Highest: Lowest Muscle Concentration*	Mean Muscle: Femoral Ratio*	S.D.*	C.V.*
1+	Temazepam	0.8–1.0	0.1	0.6	3.9	0.5	0.5–0.6	1.2	3.7	0.02	4.4
2+	Temazepam	0.8–1.0	0.8	0.8	1.1	0.8	0.4–0.9	2.0	2.0	0.1	15.8
3	Temazepam	0.8–1.0	4.3	4.7	1.1	2.2	2.0–2.6	1.3	0.5	0.2	9.1
4	Paracetamol	0.8–1.0	87	300	3.4	203	163–250	1.5	2.3	22.9	11.3
5	Paracetamol	0.8–1.0	288	341	1.2	198	122–261	2.1	0.7	46	23.2
	Prothiadin	20–92	7.9	11.8	1.5	3.1	0.4–6.0	15.0	0.4	1.8	58.1
6	Prothiadin	20–92	6.0	51.9	8.6	17.1	14.8–18.5	1.2	2.8	1.2	6.9
7+	Fluoxetine	20–42	0.3	1.1	3.3	0.3	0.03–0.6	21.7	1.0	0.2	55.9
	Thioridazine	18	0.2	1.3	6.1	0.6	0.3–1.1	3.5	2.7	0.2	40.3
8	Thioridazine	18	0.4	2.3	5.9	0.9	0.4–1.4	3.2	2.3	0.2	9.1
	Amitriptyline	6–10	1.9	12.7	6.5	4.3	1.8–8.3	4.5	2.2	1.9	43.7
9	Amitriptyline	6–10	13.1	85.0	6.5	14.8	4.9–31.2	6.3	1.1	7.2	48.7
10	Amitriptyline	6–10	1.8	24.4	13.5	10.3	8.7–13.1	1.5	5.7	1.2	11.6
11	Paracetamol	0.8–1.0	227	542	2.4	273	171–415	2.4	1.2	66.7	24.4
	Propoxyphene	16	0.9	19.8	21.3	4.0	2.0–7.3	3.6	4.3	1.73	43.7
12	Paracetamol	0.8–1.0	179	237	1.3	195	176–211	1.2	1.1	10.0	5.1
	Propoxyphene	16	1.0	9.14	9.4	3.0	2.7–3.7	1.4	3.1	0.30	10.0
13	Paracetamol	0.8–1.0	193	253	1.3	247	225–274	1.2	1.3	14.4	5.8
	Propoxyphene	16	2.5	14.4	5.8	5.4	4.3–7.9	1.8	2.2	0.98	18.1
14	Paracetamol	0.8–1.0	63	140	2.2	101	88–110	1.2	1.6	7.20	7.1
	Propoxyphene	16	1.1	6.0	5.3	3.1	1.9–3.8	2.0	2.7	0.59	19.2

Vd = volume of distribution.
S.D. = standard deviation (n − 1).
C.V. = coefficient of variance.
*does not include diaphragm.
+chronic therapeutic drug use.

avoids the hazards of calculating body drug load based upon the volume of distribution of a drug and the drug level in an autopsy blood sample. However, the tissue addition method of calculating body drug load presumes a uniformity of drug concentration within a given organ or tissue, such as muscle. The results of the present study, together with previous studies (5–7) suggest that this approach has greater limitations than previously appreciated.

The observation of variability of drug concentration within skeletal muscle also has implications in the field of entomotoxicology (14,15). Fly larvae feeding on a corpse primarily feed upon skeletal muscle. Experimental studies (16,17) have attempted to relate the drug concentration in the larvae with the drug concentration in the foodstuff. From these studies it appears that there is considerable biological variation between larvae in the extent to which they accumulate a drug. To this variability must be added the variability of the drug concentration within the skeletal muscle bulk of the corpse as evidenced by the data in this study and others (5–7). Consequently the view that fly larvae are useful for qualitative drug analysis but that quantitative interpretation of the results is extremely limited seems well founded (16,17).

In summary the data from this study reinforces the view that skeletal muscle is a useful sample for qualitative toxicological analysis but of limited quantitative value in the light of present knowledge. In exploring the potential usefulness of skeletal muscle future studies should precisely define the sampling site and provide data on peripheral muscle drug levels and peripheral blood levels. The possibility that a muscle:blood drug ratio might provide insight into the time lapse between drug ingestion and death (5) should not be discounted but rather explored more systematically. The confirmed within-case variability of drug concentrations in skeletal muscle has significant adverse implications for the calculation of total body drug load by the "tissue addition" method (12,13). The variability also reinforces the view (16,17) that drug analysis of larvae feeding on cadaver skeletal muscle has qualitative rather than quantitative value.

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