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Analysis of adulterants from cocaine preparations in lung tissue and blood

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Aims: The purity of street cocaine preparations, which are incorporated by a drug consumer, may differ considerably. Cocaine may be adulterated with a various number of pharmacologically active compounds, such as lidocaine or levamisole. Adverse side effects or lethal complications may occur if cocaine is found to contain these substances (adulterants). We examined lung tissue und blood for some typically adulterants founds in cocaine preparations and checked whether there are concentration differences in these specimens. **Methods:** The adulterants were quantified by high-performance-liquid-chromatography-time-of-flight-mass spectrometry (LC/TOF-MS) after isolation from the matrices using solid-phase (SPE) and liquid-liquid extraction (LLE). **Results and Discussion:** Phenacetin, lidocaine, diltiazem, levamisole and hydroxyzine could be found in blood and lung tissue. Levamisole could be detected in all samples. The concentrations of these substances were often higher in the lung tissue than in the correspondent body fluids. Post-mortem redistribution of the adulterants could be demonstrated. **Conclusion:** Phenacetin, lidocaine, diltiazem, levamisole and hydroxyzine accumulate in the lung and may be redistributed after death into the blood. For the interpretation of drug related deaths, adulterants of drug preparations should be taken into account in addition to the drug itself.

1. Introduction

Cocaine may be adulterated with a various number of pharmacologically active compounds, such as lidocaine or levamisole. In cases of fatal drug intoxications (FDI) the cause of death is often attributed to the drug itself, while adulterants in drug preparations are assigned only a minor role. Adverse side effects like confusion, arrhythmia or respiratory depression may occur if cocaine preparations contain these substances. We have examined lung tissue (LT), heart blood (HB) and femoral vein blood (FVB) as well as serum for 14 typical adulterants (Fig. 1) found in cocaine preparations. The aim was to find out their possible relevance in the interpretations of drug intoxications.

2. Material and Methods

2.1. Specimen

Post-mortem samples (heart blood, femoral vein blood and lung tissue) from 11 cocaine users (10 male, 1 female) and serum samples from 14 cocaine users (11 male, 3 female) were analysed.

2.2. LC-TOF/MS – sample preparation and instrumentation

The specimen were purified using automated solid-phase (bond elute certified columns) and liquid-liquid extraction methods.

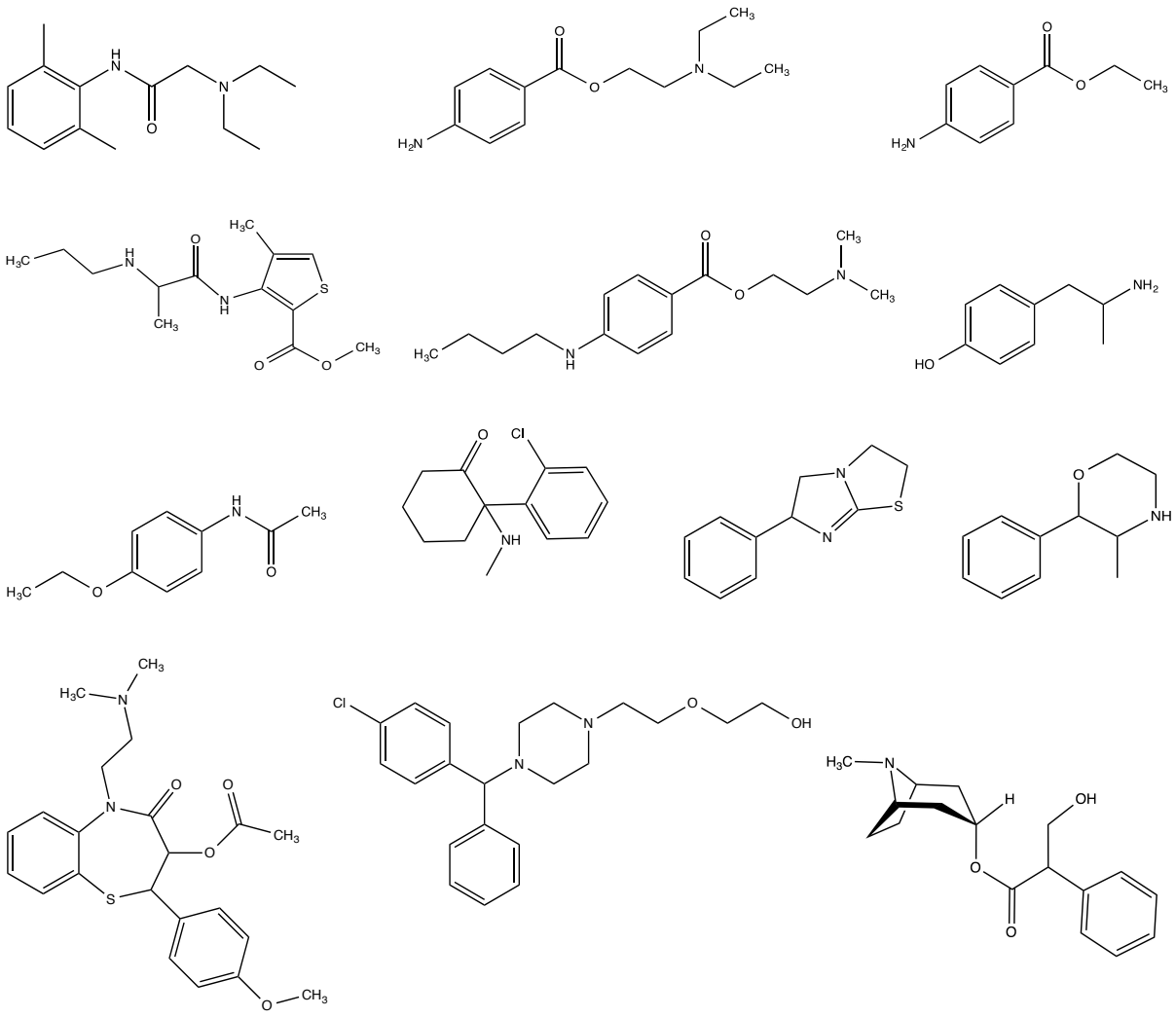

Fig. 1. Some of the typically found adulterants in cocain preparations. First line lidocaine, procaine and benzocaine among them articaine, teracaine and pholedrine, then phenacetine, ketamine, levamisole and phenmetrazine and in the last line diltiazem, hydroxyzine and atropine.

For solid-phase extraction 600 µl blood or 600 mg lung tissue, 20 µl deuterated standard mix, 100 µl water, 1 ml acetonitrile and 100 µl of isopropanol were mixed. For the liquid-liquid-extraction 600 µl blood or 600 mg lung tissue, 20 µl deuterated standard mix, 100 µl carbonate buffer (pH 8.6) and 1.2 ml of a mixture of dichloromethane/ether (70/30, v/v) were mixed. The dried extracts were mixed with 50 µl of a water/formic acid mixture. 5 µl of the final solution were analysed by LC/TOF-MS (HPLC system: Agilent 1200 series LC; mass spectrometer: Bruker Micro TOF MS-Q II).

The following conditions were used: ESI mode, YMC-Pack ODS-AQ column (150 mm x 2 mm x 3 microns) at 30°C oven temperature; full scan mode; capillary voltage 4500 V; mobile phase acetonitrile/water (containing 0.05% formic acid); flow rate of 0.2 ml/min. The evaluation was performed with the Bruker-Analysis software (Compass version 1.3 Smart Form Manually).

2.3. Validation

The method was validated using the program Valistat 2.0. The calibration for blood and lung tissue ranges from 2 - 400 ng/ml respectively ng/g. Table 1 shows the LoD and LoQ for cocaine and the adulterants in blood and lung tissue.

Tab. 1. LoD and LoQ of the method for the determination of cocaine and 9 adulterants in blood and lung tissue.

Substances	Blood [ng/ml]		Lung tissue [ng/g]	
	LoD	LoQ	LoD	LoQ
Phenacetin	0.73	2.66	0.48	1.80
Lidocaine	0.53	1.96	0.80	2.89
Diltiazem	0.77	2.79	0.85	3.06
Levamisole	0.57	2.10	0.90	3.20
Hydroxyzine	0.66	2.38	0.48	1.79
Procaine	4.15	13.8	1.59	5.55
Cocaine	1.29	4.48	2.38	7.79
Articaine	2.48	8.09	2.32	7.91
Atropine	3.86	12.7	1.27	4.39
Ketamine	1.61	5.54	1.95	6.55

3. Results and Discussion

A total of 11 cases of drug related death were collected where lung tissue (LT), heart blood (HB) and femoral vein blood (FVB) were available. In one case no femoral vein blood was available (case 4). Three of the FDI cases (cases 1, 3 and 4) were suicides whereas the other eight cases (case 2 and 5-11) were accidental drug-related deaths. In all samples some of the typically used adulterants of cocaine preparations such as levamisole, hydroxyzine, diltiazem, phenacetin or lidocaine could be detected. Furthermore cocaine and its metabolites were found in all cases, heroin was found in 9 cases and THC and its metabolites were found in 1 case (case 9). Alcohol consumption could be proven in 6 cases (cases 1 - 3, 5, 9 and 10). Additionally, other substances like caffeine, paracetamol, methadone, benzodiazepines or sildenafile were found. Procaine, benzocaine, tetracaine, pholedrine, ketamine, atropine, phenmetrazin or articaine could not be detected in any of the analysed specimens.

The diagrams (Figs. 2-6) show the concentrations of lidocaine, phenacetine, diltiazem, hydroxy-zine and levamisole in blood and LT as well as the ratios of LT/FVB (dark blue) and HB/FVB (dark red) representing the postmortem redistribution (PMR).

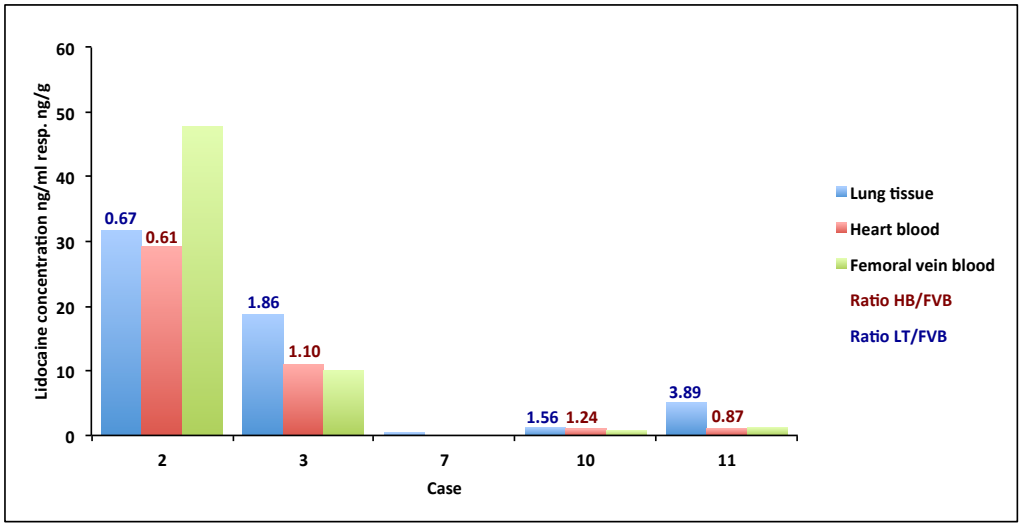

Fig. 2. Concentration of lidocaine in lung tissue (blue), heart blood (red) and femoral vein blood (green) in the cases of drug-related death positive for lidocaine. The ratios for LT/FVB and HB/FVB are shown in dark blue and dark red resp.

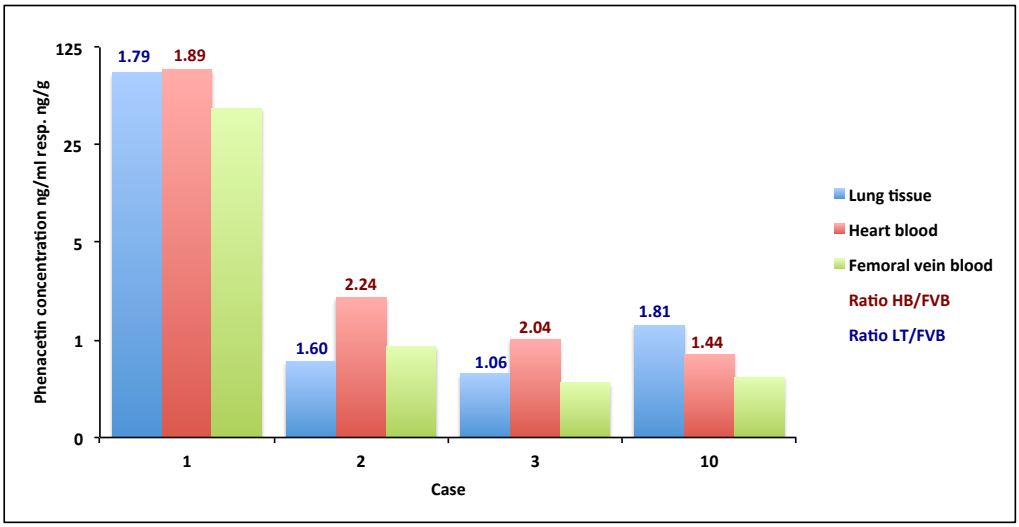

Fig. 3. Concentration of phenacetin in lung tissue (blue), heart blood (red) and femoral vein blood (green) in the cases of drug-related death positive for phenacetin. The ratios for LT/FVB and HB/FVB are shown in dark blue and dark red resp.

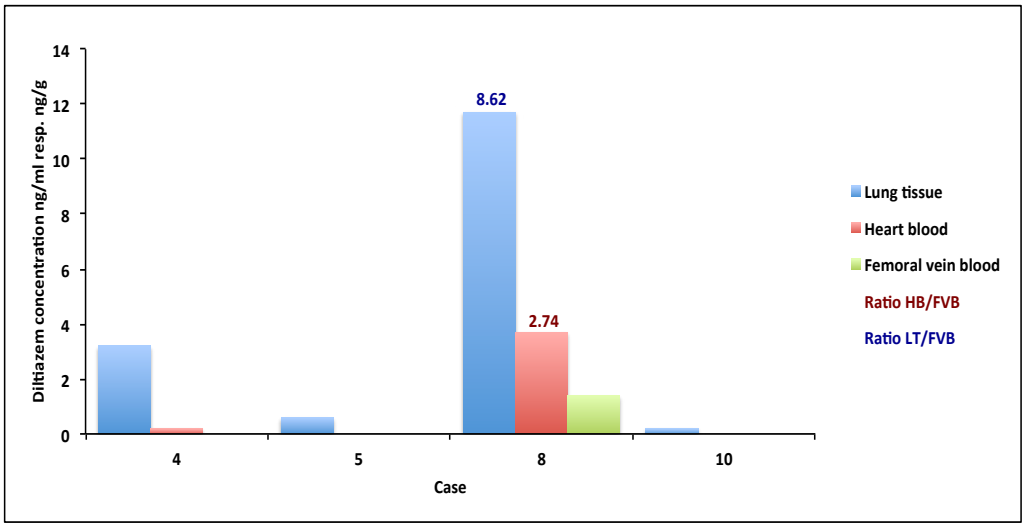

Fig. 4. Concentration of diltiazem in lung tissue (blue), heart blood (red) and femoral vein blood (green) in the cases of drug-related death positive for diltiazem. The ratios for LT/FVB and HB/FVB are shown in dark blue and dark red resp.

In the case of phenacetin, the concentrations were almost higher in heart blood than in lung tissue or femoral vein blood.

With the exception of case 1 (hydroxyzine, 31.39) the HB/FVB ratios for the other adulterants range from 0.3 to 3.6. The LT/FVB ratios were significantly higher than the HB/FVB ratios, with the exception of phenacetin in cases 1, 2 and 3. The highest LT/FVB ratio was found for hydroxyzine (case 1) with a value of 316.9. The HB/FVB as well as the LT/FVB ratios for levamisole vary widely and range from 0.3 to 3.6 (HB/FVB) and from 1.0 to 33.35 (LT/FVB) resp. The HB/FVB and LT/FVB ratios for lidocaine are with one exception (case 11; HB/FVB 0.87 and LT/FVB 3.89) nearly identical. Therefore it could be assumed that phenacetin, levamisole, diltiazem and lidocaine are redistributed at a low to moderate degree while hydroxyzine is redistributed to a high degree after death.

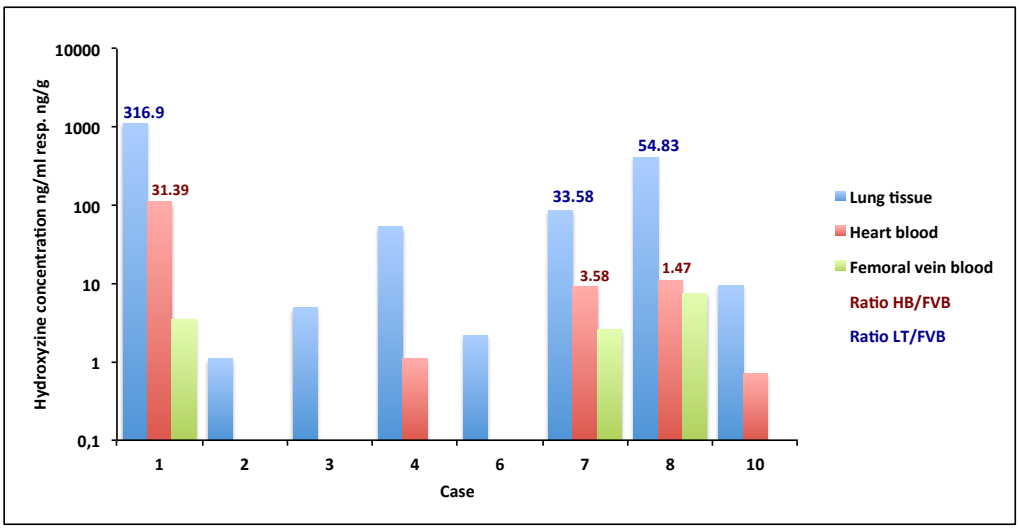

Fig. 5. Concentration of hydroxyzine in lung tissue (blue), heart blood (red) and femoral vein blood (green) in the cases of drug-related death positive for hydroxyzine. The ratios for LT/FVB and HB/FVB are shown in dark blue and dark red resp.

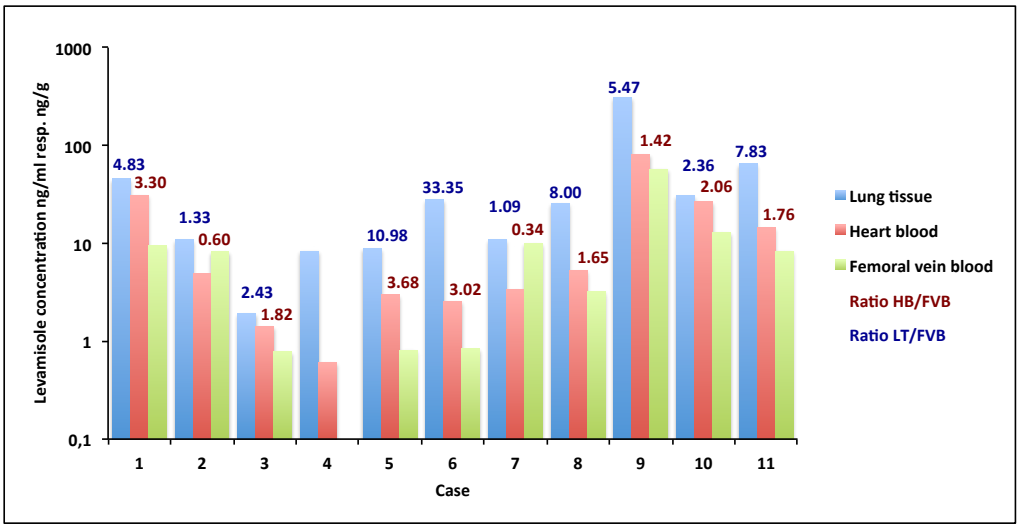

Fig. 6. Concentration of levamisole in lung tissue (blue), heart blood (red) and femoral vein blood (green) in the cases of drug-related death positive for levamisole. The ratios for LT/FVB and HB/FVB are shown in dark blue and dark red resp.

In addition 14 serum samples (7 with high and 7 with low cocaine/ benzoylecgonine concentrations) were analysed for adulterants. Phenactin (5 cases), lidocaine (3 cases), diltiazem (1 case), hydroxyzine (3 cases) and levamisole (14 cases) were detected in the samples. Beyond that methadone could be found in 7 cases, THC and its metabolites in 5 cases and alcohol in two cases. In addition some benzodiazepines could be detected. The concentrations range up to 17 ng/ml for phenacetin, up to 9 ng/ml for lidocaine, up to 3 ng/ml for hydroxyzine and up to 230 ng/ml for levamisole. Only in one sample diltiazem could be detected (6 ng/ml).

4. Conclusion

Diltiazem, phenacetin, lidocaine, hydroxyzine and levamisole are typical adulterants found in cocaine preparations. The adulterants should be quantified in blood and tissue and should be included in toxicological expertises in cases of drug related-death.

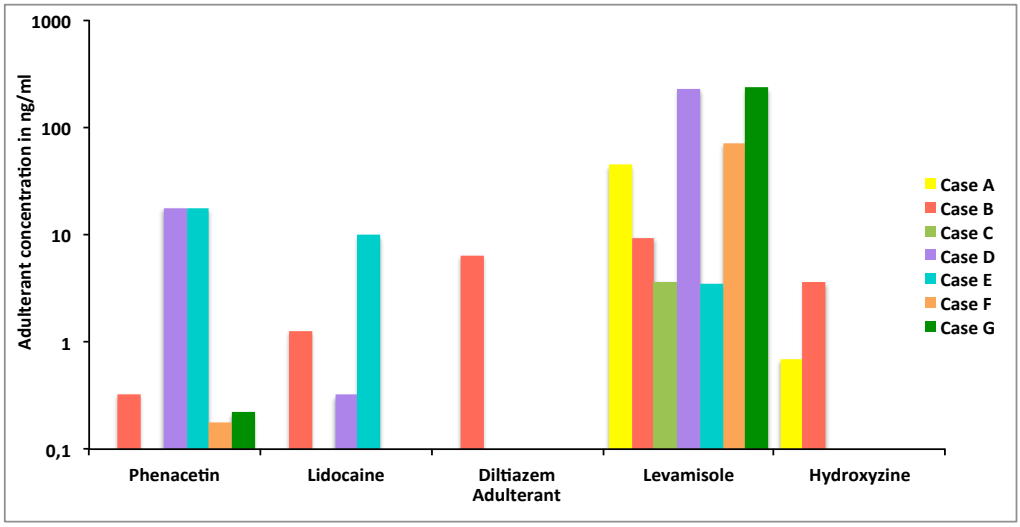

Fig. 7. The adulterants (lidocaine, phenacetin, diltiazem, hydroxyzine and levamisole) found in the serum samples of the seven cases with high cocaine / benzoylecgonine concentrations.

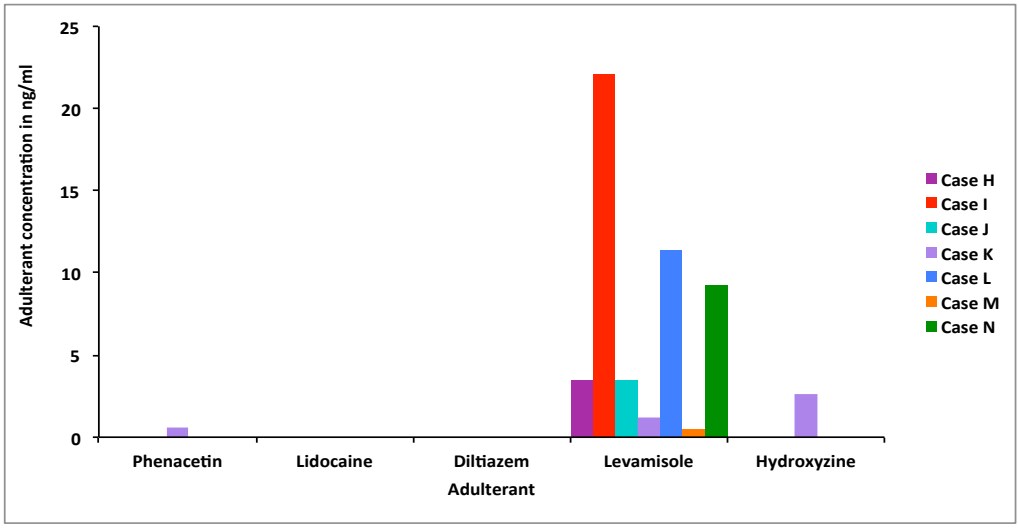

Fig. 8. The adulterants (lidocaine, phenacetin, diltiazem, hydroxyzine and levamisole) found in the serum samples of the seven cases with low cocaine / benzoylecgonine concentrations.

Phenacetin, levamisole, diltiazem and lidocaine are redistributed to a moderate degree, while hydroxyzine seems to be extensively redistributed after death.

5. References

For further information and all references see the article: Drug-related death: Adulterants from cocaine preparations in lung tissue and blood, published in Forensic Science International 2015;249:294-303.