Received: 3 September 2021 | Revised: 8 April 2022 | Accepted: 16 April 2022

DOI: 10.1111/bcp.15365

ORIGINAL ARTICLE

Concentrations of psychoactive substances in blood samples from non-fatal and fatal opioid overdoses

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Funding information
The study was partly funded by the National Overdose Strategy. The funder was not involved in the planning of the study design or in the collection, analysis or interpretation of data or in the writing of the manuscript.

Aim: The primary aim was to compare concentrations of psychoactive substances in blood in non-fatal and fatal opioid overdoses. The secondary aim was to assess the concentration levels of naloxone in blood in non-fatal overdoses and the association between naloxone findings and concomitantly detected drugs.

Method Design: Case-control study.

Setting: Norway. Fatal overdoses from 2017 and non-fatal overdoses from February 2018 to September 2019.

Cases: Thirty-one non-fatal and 160 fatal opioid overdose cases. Data from the non-fatal overdoses were collected from hospital records and blood samples, and data from the fatal overdoses were collected from autopsy reports. Concentrations of psychoactive substances (including ethanol) in blood samples were collected at the time of hospital admission for the non-fatal overdoses and during autopsy for the fatal overdoses.

Results: The median number of different substances detected was four for fatal and five for non-fatal overdoses. The fatal overdoses had higher pooled concentrations of opioids (188 *vs* 57.2 ng/mL, $P < .001$), benzodiazepines (5467 *vs* 2051 ng/mL, $P = .005$) and amphetamines (581 *vs* 121 ng/mL, $P < .001$) than the non-fatal overdoses. A linear relationship between naloxone and concomitant pooled opioid concentrations was found (95% confidence interval = 0.002-0.135, $P < .05$).

Conclusion: The total load of drug concentrations was associated with the fatal outcome of an overdose, while the number of drugs used, to a lesser extent, differentiated between those who survived and those who died from an overdose. Higher opioid concentrations were associated with treatment with higher naloxone doses.

KEYWORDS
amphetamines, benzodiazepines, drug concentrations, naloxone, non-fatal overdose, opioid overdose, opioids, overdose death

1 | INTRODUCTION

Overdose from opioid use is a significant global health problem. In 2018, an estimated 58 million people used opioids and more than 110 000 opioid-related deaths occurred.[1] High overdose death rates have been reported in Norway, and from 2015 to 2019 the annual number was approximately five per 100 000 inhabitants.[2] More than 80% of overdose deaths in Norway are opioid-related, most often in combination with four to five other psychoactive substances.[3–5] In comparison, the United States has experienced an opioid epidemic with an extremely high number of opioid-related overdose deaths. Their rate is 14 per 100 000 inhabitants,[6] which is three times the rate in Norway. Synthetic opioids, such as fentanyl and fentanyl analogues, have contributed strongly to these numbers.

Current knowledge about the cause of death in overdoses is mainly based on autopsy findings,[7] despite the fact that the incidence of non-fatal overdoses is significantly higher than the incidence of fatal overdoses.[8] It has been estimated that approximately 5% of heroin overdoses are fatal.[9] Risk factors for fatal overdoses are concomitant use of opioids with other psychoactive drugs, intravenous administration and previous overdoses. More knowledge about how drug use prior to non-fatal overdoses differs from fatal overdoses would enhance our understanding of these risk factors. Comparing drug concentrations from non-fatal and fatal opioid overdoses could therefore provide useful information.

Two Norwegian studies have investigated drug concentrations based on drug findings in autopsy samples. The first study compared drug concentrations in blood samples from fatal intoxications with apprehended drivers suspected of driving under the influence of drugs.[5,10] The second study compared deceased patients using opioid maintenance treatment (OMT), where one group died due to intoxications and the other group died from other causes.[11] To our knowledge, there is limited literature on comparisons between drug concentrations in blood from non-fatal overdoses and fatal overdoses.

To reduce the opioid toxicity and hence the number of fatal overdoses, naloxone is administered as an antidote by emergency medical services personnel and bystanders in out-of-hospital settings in Norway.[12] Naloxone concentrations in blood have previously been studied in relation to dose and route of administration,[13] but there is limited knowledge concerning naloxone concentrations in blood after administration for opioid overdoses.

1.1 | Aims

The purpose of the study was to compare concentrations of the most commonly detected psychoactive substances and pooled estimates of opioids and benzodiazepines in blood in non-fatal (2018-2019) and fatal (2017) opioid overdoses. We further wanted to assess naloxone concentration levels and the association between naloxone findings and concomitantly detected substances in blood in non-fatal opioid overdoses.

What is already known about this subject

- Opioid and polydrug use is common among individuals who experience overdoses.
- In opioid maintenance treatment, overdose death cases have higher opioid concentrations than patients who die from other causes.
- Approximately 5% of overdoses are fatal.

What this study adds

- The number of substances detected did not appear to make a difference between survival and fatal outcome in cases of overdose.
- Fatal opioid overdose cases had higher concentrations of opioids, benzodiazepines and amphetamines than non-fatal opioid overdose cases.
- Among the nonfatal overdoses, naloxone was detected in significantly higher concentrations when the pooled opioid concentration increased.

2 | METHODS

2.1 | Study design and setting

This was a case-control study using data from two populations: non-fatal and fatal opioid overdoses. Data from the non-fatal overdoses were collected from hospital records and blood samples after patient admission to Oslo University Hospital (OUH), Norway, while data from nationwide fatal overdoses in Norway were collected from autopsy reports and forensic toxicological analyses.

2.2 | Participants

2.2.1 | Non-fatal opioid overdoses

The paramedics assessed the patients and those classified with an opioid overdose according to anamnestic information, clinical signs and symptoms were offered admission to the Section for Emergency Addiction Services and Detoxification at the Department on Substance Use Disorder Treatment, OUH. Patients were included from February 2018 to September 2019. Only one patient was unable to participate.

On admission, a 5-mL BD Vacutainer Plastic Fluoride/Oxalate tube of whole blood was collected for analyses of ethanol, illicit drugs, psychoactive medicines and fentanyl analogues (see Table 1 for

TABLE 1 Included drugs and their cut-off concentrations in blood

Analyte	Drug group classification for statistical purposes	Cut-offs (ng/mL)
6-monoacetylmorphine (6-AM)[a]	Opioid	10
Buprenorphine	Opioid	0.4
Codeine[a]	Opioid	9.0
Fentanyl[a]	Opioid	0.7
Methadone	Opioid	16
Morphine	Opioid	8.6
Oxycodone	Opioid	11
Tramadol	Opioid	53
Naloxone[b]	Opioid antagonist	0.01
Alprazolam	Benzodiazepine	3.1
Clonazepam (+ the metabolite 7-aminoclonazepam among the deceased)	Benzodiazepine	1.3
Diazepam	Benzodiazepine	57
Flunitrazepam	Benzodiazepine	1.6
Nitrazepam	Benzodiazepine	2.8
Oxazepam	Benzodiazepine	5.7
Zolpidem[c]	Benzodiazepine	22
Zopiclone[c]	Benzodiazepine	7.8
Amphetamine	Amphetamines	27
Methamphetamine	Amphetamines	30
3,4-methylenedioxymethamphetamine (MDMA/ecstasy)	Illicit stimulant/hallucinogenic drug	39
Cocaine	Illicit stimulant	15
Benzoylecgonine	Metabolite from cocaine	58
Δ9-tetrahydrocannabinol (THC)	Cannabis	0.6
Alimemazine	Antihistamine	15
Hydroxyzine	Antihistamine	75
Amitriptyline	Antidepressant	56
Levomepromazine	Antipsychotic drug	33
Quetiapine	Antipsychotic drug	23
Gabapentin	Pregabalin and/or gabapentin	17
Pregabalin	Pregabalin and/or gabapentin	796
Lamotrigine	Neuroleptic drug	256
Ethanol	Alcohol	0.095 g/kg

Note: The included fentanyl analogues are described elsewhere.[14]

[a]Prevalence of opioids also includes 6-AM, codeine and fentanyl, which lack morphine equivalent conversion factors.

[b]Naloxone was not included in the analyses of the postmortem samples.

[c]The z-hypnotic drugs zolpidem and zopiclone are considered as benzodiazepines because of their similar pharmacological effects to benzodiazepines.

details), collected simultaneously with the diagnostic blood samples. The samples were stored in a refrigerator until analysis, a maximum of 1 week later.

Additional recorded variables were sex, age, time of overdose, naloxone administration, blood sampling, self-reported use of psychoactive drugs and route of administration.

2.2.2 | Fatal opioid overdoses

Nationwide fatal opioid-related overdoses in the period between 1 January 2017 and 31 December 2017 were included. Original inclusion criteria, methodology and analytical findings have previously been published.[3] In the present study, fatal overdoses were included

TABLE 2 Conversion factors for the pooled opioids and benzodiazepines

Opioids	Benzodiazepines
Morphine[a] 1.00	Diazepam[a] 1.00
Buprenorphine 37.5	Alprazolam 20.0
Methadone 0.375	Clonazepam[c] 48.0
Oxycodone[b] 0.60	Nitrazepam 3.30
Tramadol 0.20	Oxazepam 0.33
	Zolpidem[b] 2.00
	Zopiklone[b] 6.70

[a]Did not include any metabolites.
[b]Not detected above cut-off among the overdose survivors.
[c]The metabolite 7-aminoclonazepam was included and multiplied by 48 among the lethal overdose cases to account for postmortem changes of clonazepam which reduce the concentration after death.

if the main intoxicant was one of the opioids mentioned in Table 1 or one of the fentanyl analogues described elsewhere.[14]

The present study recorded age, sex, manner of death, main intoxicant, poly drug intoxication (yes/no) and signs of resuscitation (yes/no/unknown).

2.3 | Measurements

The relative potencies of different opioids and benzodiazepines and the principle of equipotent doses were applied for comparisons of the concentration of opioids and benzodiazepines.[15] We used conversion factors already implemented in the Norwegian Road Traffic Act for drug concentrations in blood to estimate pooled morphine- and diazepam-equivalent concentrations of opioids and benzodiazepines.[16] In addition, we included buprenorphine and tramadol, and we assumed that the conversion factors for their concentrations in blood are similar to their conversion factors for equipotent doses.[17] Table 2 presents the included drugs and their conversion factors. These conversion factors have been used in previous studies of drug concentrations among overdose cases and other known drug users.[5,11] We therefore assume that a comparison with alcohol intoxication affecting driving ability and intoxication leading to overdose is reasonable.

2.4 | Substances

2.4.1 | Non-fatal opioid overdoses

Ethanol was analysed in blood samples using an enzymatic alcohol dehydrogenase method.[18] Ultra-high pressure liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) was used for the analyses of 34 other psychoactive substances[19] (Table 1), and 27 fentanyl analogues and naloxone (described in detail in a previous paper[14]). The analyses were conducted by the Department of Forensic Sciences at OUH.

2.4.2 | Fatal opioid overdoses

The fatal opioid overdose cases were investigated by forensic autopsy, including toxicological analyses in post-mortem whole-blood samples from a peripheral vein.[3] The analytical repertoire consisted of more than 100 drugs and ethanol, and details regarding the procedures are described elsewhere.[5] In addition, the department uses an ultra-high-pressure chromatography-q-TOF instrument for an expanded search of a high number of drugs, including fentanyl and fentanyl analogues. Positive findings were confirmed and quantified using UHPLC-MS/MS.[14]

2.4.3 | All data

Commonly used opioids, benzodiazepines and other psychoactive drugs included in the analytical repertoire of both groups were studied. Table 1 gives an overview of the included analytes, their drug group classification (for statistical purposes) and the common cut-off concentrations applied in this study. The included fentanyl analogues are presented elsewhere.[14]

2.5 | Statistical analysis

Data are presented as means, standard deviations (SD), frequencies and proportions. Two-sided Student's *t*-test was used to compare continuous data and the chi-square test was used for categorical data. The drug concentrations were not normally distributed and are therefore presented with median and interquartile range values. A Mann-Whitney U test was used for comparisons. Bivariate and multiple regression models were run to assess the association between overdose deaths and pooled opioid and benzodiazepine concentrations and concentrations of amphetamines, with the non-fatal overdoses as the reference category. Sex and age were included in all models, whereas different combinations of pooled opioid, pooled benzodiazepine and amphetamine concentrations were included in four different multiple models. The results were presented as odds ratios (ORs) with 95% confidence intervals (CIs). Only cases with no missing covariate values were included in the multiple models. Furthermore, due to the wide concentration range for pooled benzodiazepines, this variable was rescaled in the regression model (divided by 1000 and presented as pg/mL). Linear regression was performed to study associations between naloxone concentrations and time between administration and blood sampling, and associations between pooled opioid and benzodiazepine concentrations. All analyses were two-sided and the significance level was set as $P < .05$. Data were analysed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

2.6 | Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics South-East (case numbers 2017/2474 and

TABLE 3 Sample characteristics, number of detected drugs and prevalence of detected opioids, benzodiazepines, Δ9-tetrahydrocannabinol (THC), amphetamines, pregabalin and/or gabapentin, and ethanol

	All (n = 191)	Deceased (n = 160)	Overdose Survivors (n = 31)	*P* value
Age, mean ± D	40.5 ± 11.9	41.2 ± 12,0	36.7 ± 11.1	0.052[a]
Men, n (%)	141 (76)	120 (75)	21 (68)	0.400
Median number of psychoactive drugs detected	4.0	4.0	5.0	0.150[a]
Opioids detected, n (%)	185 (97)	159 (99)	26 (84)[a]	<0.001
Benzodiazepines detected, n (%)	164 (86)	133 (83)	31 (100)	0.014
THC detected, n (%)	69 (36)	61 (38)	8 (26)	0.191
Amphetamines detected, n (%)	68 (36)	46 (29)	22 (71)	< 0.001
Pregabalin and/or gabapentin, n (%)	39 (20)	39 (24)	0 (0)	0.002
Ethanol detected, n (%)	13 (6.8)	10 (6.3)	3 (9.7)	0.488

[a]*t*-test, equal variances assumed.

2017/2396) and by the Director of Public Prosecution. As many patients were too intoxicated to be able to consent at the time of admission, it was accepted that we obtained active consent after the sample was taken.

3 | RESULTS

3.1 | Characteristics

Thirty-one non-fatal and 160 fatal opioid overdoses were included. The mean age was somewhat lower among the non-fatal than the fatal overdoses (mean age 36.7 SD ± 11.1 *vs* 41.2 SD ± 12.0, $P = .052$). There was no significant difference in the sex distribution between the two overdose groups. Furthermore, the non-fatal overdoses had a higher median number of detected psychoactive drugs in the blood than the fatal overdoses (five versus four; Table 3). The median concentrations were in general much higher among the fatal overdoses (Table 4). None of the non-fatal overdoses had only a single compound in their blood at the time of hospital admission (Figure 1). Among the overdose deaths, only 3% (n = 5) died from mono-intoxications. The intoxicants were fentanyl, carfentanil, methadone and tramadol in these cases.

3.2 | Drug concentrations

Opioids included in the pooled estimation were detected in 84% of the non-fatal overdoses and 96% of the fatal overdoses (Table 4). The pooled opioid concentration was significantly higher among the fatal overdose cases (188 *vs* 57.2 ng/mL fatal to non-fatal cases, respectively, $P < .001$; Table 4). An opioid, including fentanyl and fentanyl analogues, was detected in 99% of the overdose deaths ($P < .001$). Fentanyl and fentanyl analogues were not detected in the non-fatal overdose group. Overall, morphine was the most frequent opioid detected. The median concentration was significantly higher among the fatal overdoses (131 *vs* 47.4 ng/mL fatal to non-fatal cases, respectively, $P = .001$). The second most frequent opioid was methadone. The median concentration was significantly higher among the fatal overdoses (151 *vs* 21.0 ng/mL fatal to non-fatal cases, respectively, $P = .005$). Fentanyl analogues were seldom detected and only one fatal overdose case from carfentanil occurred.

Benzodiazepines were detected in 100% of the non-fatal and 83% of the fatal overdoses (Table 4). The pooled concentration was, however, significantly higher among the fatal overdose cases (5467 *vs* 2051 ng/mL fatal to non-fatal cases, respectively, $P = .005$; Table 4). The most frequent benzodiazepine was clonazepam, which was detected among 90% of the non-fatal overdose cases and 57% of the fatal overdoses. The concentration of clonazepam was significantly higher among the fatal overdose cases despite the lower prevalence (8502 *vs* 2220 ng/mL fatal to non-fatal cases, respectively, $P < .001$). Diazepam, the second most frequent benzodiazepine, was detected in 26% of the overdose deaths. The concentration was significantly lower among the fatal overdose cases than the non-fatal cases (119 *vs* 240 ng/mL fatal to non-fatal cases, respectively, $P = .004$).

Among the other drugs analysed, amphetamines and Δ9-tetrahydrocannabinol (THC) were the most frequently detected. Amphetamines were detected in 71% of the non-fatal overdoses compared with only 29% of the blood samples from the overdose deaths. The median concentration, however, was significantly higher among the fatal overdoses (581 *vs* 121 ng/mL fatal to non-fatal cases, respectively, $P < .001$). THC was seen in 26% of the non-fatal overdoses and 38% of the fatal overdose cases, and the concentrations were not significantly different between the two groups. Ethanol was the third most frequently detected other substance, found in 10% of the non-fatal and 6% of the fatal overdoses, and in significantly higher levels among the fatal overdoses (1.45 *vs* 0.76 g/kg, $P = .028$). Pregabalin was detected in 18% of the fatal overdoses, but not among the overdose survivors.

TABLE 4 Substances detected in blood from non-fatal and fatal opioid overdoses, with median (interquartile range, IQR) concentrations (ng/mL)

	All, n = 191		Deceased, n = 160		Overdose survivors, n = 31		Mann-Whitney *U* test
	N (%)	Median (IQR) concentration ng/mL	N (%)	Median (IQR) concentration ng/mL	N (%)	Median (IQR) concentration ng/mL	*P*
Pooled opioids[a]	**179 (94)**	**160 (272)**	**153 (96)**	**188 (321)**	**26 (84)**	**57.2 (64.2)**	***P* < .001**
Morphine	99 (52)	94.2 (177)	76 (48)	131 (210)	23 (74)	47.4 (63.9)	*P* = .001
Methadone	62 (32)	126 (229)	57 (36)	151 (224)	5 (16)	21.0 (60.3)	*P* = .005
Buprenorphine	32 (17)	43.7 (82.0)	30 (19)	44.5 (127)	2 (6.5)	21.9 (–)	*P* = .121
Oxycodone	19 (9.9)	241 (285)	19 (12)	241 (285)	0	…	…
Tramadol	14 (7.3)	229 (1191)	14 (8.8)	229 (1191)	0	…	…
Pooled benzodiazepines[a]	**164 (86)**	**4271 (9738)**	**133 (83)**	**5467 (11462)**	**31 (100)**	**2051 (2476)**	***P* = .005**
Clonazepam	119 (62)	5793 (11038)	91 (57)	8502 (12512)	28 (90)	2220 (2093)	*P* < .001
Diazepam	43 (23)	137 (138)	35 (22)	119 (108)	8 (26)	240 (166)	*P* = .004
Alprazolam	40 (21)	648 (1276)	32 (20)	843 (1242)	8 (26)	156 (656)	*P* = .060
Oxazepam	30 (16)	76.8 (149)	21 (13)	109 (157)	9 (29)	10.9 (30.7)	*P* = .001
Nitrazepam	8 (4.2)	78.3 (283)	5 (3.1)	63.9 (362)	3 (9.7)	92.8 (–)	*P* = .786
Zopiclone	16 (8.4)	339 (3420)	16 (10)	339 (3417)	0	…	…
Zolpidem	2 (1.0)	771 (–)	2 (1.3)	771 (–)	0	…	…
Other							
Fentanyl[b]	9 (4.7)	10.8 (17.3)	9 (5.6)	10.8 (17.3)	0	…	…
Δ9-tetrahydrocannabinol	69 (36)	2.33 (3.92)	61 (38)	2.33 (4.06)	8 (26)	1.81 (1.33)	*P* = .330
Amphetamines	68 (36)	324 (810)	46 (29)	581 (1124)	22 (71)	121 (224)	*P* < .001
Ethanol, g/kg	13 (6.8)	1.24 (1.04)	10 (6.3)	1.45 (1.21)	3 (9.7)	0.76 (–)	*P* = .028
Quetiapine	10 (5.2)	142 (555)	8 (5.0)	192 (899)	2 (6.5)	42.8 (–)	*P* = .178
Pregabalin	29 (15)	5573 (6130)	29 (18)	5573 (6130)	0	…	…
Gabapentin	11 (5.8)	11 302 (10103)	11 (6.9)	11 302 (10103)	0	…	…

[a]Pooled opioids and pooled benzodiazepines are those mentioned in Table 2 with established morphine- and diazepam-equivalent conversion factors, respectively.
[b]Fentanyl was not included among the pooled opioids and is therefore included as "other" in this table.

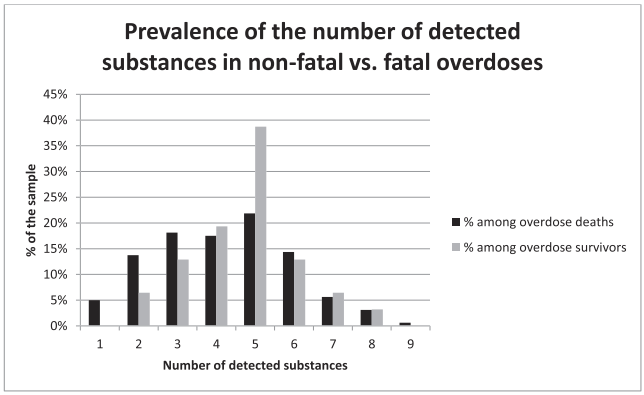

FIGURE 1 Distribution of number of detected drugs among non-fatal and fatal opioid overdoses

3.3 | Factors associated with overdose death

Table 5 shows the results from bivariate analyses and four multiple regression models assessing covariates associated with overdose death compared with overdose survival. Age and sex were included in all models, without any significant associations being found. In bivariate analyses, pooled opioid concentration, pooled benzodiazepine concentration and amphetamine concentration were associated with higher odds of fatal overdose. Without amphetamines in the multiple model, pooled opioids (OR = 1.02, CI = 1.01-1.03, *P* < .001) and pooled benzodiazepines (OR = 1.25, CI = 1.06-1.47, *P* = .007) were associated with higher odds of fatal overdose (model 1). This means that one unit increase in pooled opioid concentration (eg, from 57.2 to 58.2 ng/mL) is associated with a 2% increased risk of dying, and

TABLE 5 Factors associated with fatal versus non-fatal (reference) opioid overdose

Model	Bivariate models OR (95% CI)	Multiple model 1[a] OR (95% CI)	Multiple model 2[b] OR (95% CI)	Multiple model 3[c] OR (95% CI)	Multiple model 4[d] OR (95% CI)
Age					
<40	1	1	1	1	1
≥40	1.34 (0.62-2.91)	0.85 (0.29-2.47)	0.90 (0.17-4.84)	1.15 (0.18-7.31)	0.98 (0.22-4.31)
Sex					
Men	1	1	1	1	1
Women	0.70 (0.30-1.61)	0.83 (0.26-2.64)	0.12 (0.01-1.97)	0.21 (0.01-4.71)	0.45 (0.06-3.39)
Pooled opioid concentration in ng/mL (continuous)	1.02 (1.01-1.03)**	1.02 (1.01-1.03)**	1.02 (1.00-1.05)*	1.01 (0.99-1.04)	…
Pooled benzodiazepine concentration[e] in pg/mL (continuous)	1.16 (1.05-1.29)*	1.25 (1.06-1.47)*	…	1.16 (0.91-1.49)	1.26 (1.03-1.54)*
Amphetamines, ng/mL (continuous)	1.00 (1.00-1.01)*	…	1.00 (1.00-1.01)*	1.01 (1.00-1.01)	1.00 (1.00-1.01)*

Abbreviations: CI, confidence interval; OR, odds ratio.
[a]Only complete cases are included in the multiple model, $N = 155$.
[b]Only complete cases are included in the multiple model, $N = 65$.
[c]Only complete cases are included in the multiple model, $N = 60$.
[d]Only complete cases are included in the multiple model, $N = 62$.
[e]Due to the wide concentration range for pooled benzodiazepines, this variable was rescaled in the regression model (divided by 1000).
**$P < .001$, *$P < .05$.

one unit increase in pooled benzodiazepine concentration (eg, from 2.051 to 3.051 pg/mL, ~2051 to 3051 ng/mL) is associated with a 25% increased risk of dying. The model also adjusted for age, sex and pooled opioid- and benzodiazepine concentration, respectively. Multiple model 2 included pooled opioids and amphetamines, with significant association between concentration level and fatal opioid overdose (pooled opioids OR = 1.02, CI = 1.00-1.05, $P = .032$; amphetamines OR = 1.00, CI = 1.00-1.01, $P = .046$). When concentrations of pooled opioids, pooled benzodiazepines and amphetamines were included in the same model, the significance level was $P > .05$ for all variates. Finally, model 4 included pooled benzodiazepines and amphetamines, with significant results for both drug groups (pooled benzodiazepines OR = 1.26, CI = 1.03-1.54, $P = .028$; amphetamines OR = 1.00, CI = 1.00-1.01, $P = .007$).

3.4 | Drug group combinations

Opioids and benzodiazepines were found simultaneously in more than 80% of each opioid overdose group. Opioids and amphetamines were detected more often among the non-fatal overdoses (65% *vs* 28%).

3.5 | Naloxone

Naloxone was administered to 22 of the 31 non-fatal overdoses (Table 6), but was only detected in blood from 15 of them (68%). One additional patient had naloxone in the blood with no hospital record of the administration. Naloxone was not in the analytical repertoire among the overdose deaths.

There was a linear relationship between blood concentrations of naloxone and concomitant pooled opioid concentrations (95% CI = 0.002-0.135, $P < .05$), but not for pooled benzodiazepine concentrations. The numbers of psychoactive substances found in blood were equal in the cases with and without detected naloxone (4.8 *vs* 4.6, $P = .679$).

4 | DISCUSSION

In the present study, the fatal overdoses had higher pooled opioid, pooled benzodiazepine and amphetamine concentrations in blood than the non-fatal overdose cases. The fatal opioid overdose cases had similar median concentrations to the overdose deaths studied previously.[5,11] There were no statistically significant differences regarding age and sex between the two opioid overdose groups. Both groups included polydrug users and the number of different drugs detected was somewhat higher in the non-fatal overdose group than among the deceased.

Bech et al found median pooled opioid concentrations of 362 ng/mL among drug-induced deaths in OMT patients,[11] and Edvardsen et al found 211 ng/mL in general overdose deaths and 225 ng/mL in heroin/morphine-positive overdose deaths.[5,10] These results are comparable with the median concentration among fatal overdose cases in the present study. The pooled median benzodiazepine concentration among the fatal overdoses was at the higher end of Bech et al's and Edvardsen et al's previously reported findings.[5,10,11] The non-fatal overdoses in the present study had, in comparison, a substantially lower median pooled benzodiazepine concentration than the fatal opioid overdoses. Amphetamines were also detected frequently, and

TABLE 6 Cases with reported time (minutes) from naloxone administration with corresponding naloxone concentration, pooled opioid concentration, pooled benzodiazepine concentration, detected opioids and total number of drugs detected in blood

Case	Minutes from naloxone administration to blood sampling	Naloxone concentration (ng/mL)	Pooled opioid concentration (ng/mL)	Pooled benzodiazepine concentration (ng/mL)	Detected opioids	Number of psychoactive drugs detected above cut-off
1	43	0.25	28.0	636	Morphine	3
2	50	Not detected	72.5	3349	Morphine	5
3	62	0.53	94.2	14 108	6-AM below cut-off, morphine	4
4	70	5.57	117	296	6-AM below cut-off, codeine, morphine	5
5	85	Not detected	198	501	Methadone, morphine	4
6	100	0.95	66.8	7046	Morphine	3
7	105	0.60	64.0	3076	Methadone, morphine	4
8	105	0.58	113	1048	Codeine, morphine	5
9	108	Not detected	31.2	979	Morphine	5
10	110	0.09	29.4	7986	Morphine	5
11	133	0.43	51.9	1940	Morphine	4
12	135	Not detected	29.3	3793	Morphine	5
13	135	0.02	62.5	1697	Methadone, morphine	6
14	135	0.90	93.3	3184	Codeine, morphine	6
15	155	0.36	99.6	2562	Codeine, morphine	7
16	160	Not detected	4.00	5061	…	2
17	168	0.27	6.85	273	…	3
18	175	0.39	44.0	57.6	Morphine	4
19	277	0.20	22.3	1727	Morphine	6
20	295	Not detected	44.0	830	6-AM, morphine	5
21	330	0.63	35.7	3524	Morphine	5
22	1226	Not detected	4.00	1201	…	4
23	Unknown	1.02	75.7	2724	Codeine, morphine	7
24	…	…	28.1	2051	Buprenorphine	8
25	…	…	31.1	1849	Morphine	5
26	…	…	97.3	3036	Methadone	6
27	…	…	79.8	1288	Methadone, morphine	5
28	…	…	15.8	4334	Buprenorphine	5
29	…	…	24.8	1961	Morphine	5
30	…	…	…	2533	…	3
31	…	…	…	5366	…	2

Abbreviation: 6-AM, 6-monoacetylmorphine.

their contribution to overdose death is uncertain and beyond the scope of the present study. In the present study, the median concentration of amphetamines in the overdose deaths was higher than previously reported.[5,10] Edvardsen et al found the median concentration was 386 ng/mL in overdose deaths[5,10] and Bech et al reported a median concentration in blood of 365 ng/mL in a group of drug-induced deaths. The non-fatal overdoses in the present study had a median concentration of amphetamines that was lower than the reported amphetamine concentrations mentioned.

Possible causes for higher drug concentrations among the overdose deaths are larger drug intake, higher purity of the drugs and other administration routes. Intravenous administration gives, in general, higher maximum concentration after intake than the same dose given by other administration routes. A recent study showed that amount and type of acid used and the heating conditions during the preparation of heroin affect the potency of the final injected solution.[20] Higher drug intake will produce higher concentrations than lower doses of the same drug, but there can be large inter-individual

differences due to pharmacokinetic factors such as genetic polymorphisms[21] that alter the bioavailability and metabolism. Recent studies showed that Norwegian drug abusers inject other drugs besides heroin, such as amphetamines.[22,23] The outcomes after amphetamine toxicity depend on the number of organs affected, the dose ingested, administration of any potent decongestants and patient comorbidities.[24] Amphetamines might reduce the respiratory depressant effect of opioids,[25] and our study shows that identification of mechanisms by which amphetamines interfere with the probability of overdosing is needed and of great importance in overdose prevention.

The self-reported data from the non-fatal overdose cases showed that most patients injected drugs prior to the present overdose, indicating that the fatal overdoses might have used higher dosages or drugs with higher purity, possibly because the user had developed opioid tolerance. Certain preparation methods of heroin, such as adding acid and heat, could also cause higher concentrations of 6-monoacetylmorphine (6-AM) and morphine.[20] Postmortem drug changes in blood concentrations cannot be completely excluded as a contributing factor for elevated concentrations either, but Bech et al's study reported concentration differences between two groups of deceased OMT patients.[11] Our findings, supported by previously reported results, show that increasing concentrations of pooled opioids and pooled benzodiazepines, respectively, are of large relevance in overdose deaths. In addition, the combination of pooled opioids and pooled benzodiazepines increases the death risk in opioid overdoses. Their sedative effects on the respiratory system are an important reason for this. Increasing concentrations of amphetamines are probably important as well as they were apparent among the fatal opioid overdoses.

The non-fatal overdose cases were not treated with opioids or benzodiazepines as long as they were under the supervision of healthcare professionals. It is not likely that these drugs interfered in the fatal overdoses either. Some of the fatal overdose cases (30%) had information about, or signs of, resuscitation attempts in the autopsy report, but opioids and benzodiazepines were not administered as part of medical aid in these overdoses. Amphetamines have a very low prevalence of medicinal use in Norway, and it is not likely that they were present as a result of medical treatment.

Because naloxone was not included in the drug repertoire within forensic toxicological analyses, we do not know whether more non-fatal overdoses were treated with naloxone than the overdose deaths. This also needs further investigations, and we are currently including naloxone in the drug repertoire at the forensic toxicology laboratory in autopsy cases. Because of Norwegian regulations, we are not allowed to reanalyse biological material from cases where naloxone was not considered during routine analyses of the case. All of the non-fatal overdose patients were observed by or received medical treatment from ambulance workers and other healthcare professionals, which might have saved their lives. We do not know the extent of medical aid in the overdose deaths. Furthermore, a linear relationship between blood concentration of naloxone and concomitant pooled opioid concentration was found, which indicates correct treatment with higher doses of naloxone in what could be more opioid-affected and sedated patients. The short half-life of naloxone (1-1.5 hours) might explain why naloxone was not detected in all of the naloxone-treated patients.

4.1 | Strengths and limitations

This study adds useful information on the concentrations of pooled opioids and pooled benzodiazepines in blood from non-fatal and fatal opioid overdose cases, which is sparsely covered in the literature. The present study also included the most common psychoactive drugs[3,26] and used an identical analytical repertoire and cut-off concentrations to be able to make comparisons between the groups. The exception is the interpretation of concentrations of clonazepam/7-aminoclonazepam, which is difficult in the autopsy cases due to postmortem degradation of clonazepam and the formation of 7-aminoclonazepam.[27] No overdoses with main intoxicants other than opioids were included in the study, making the two groups as equal as possible for comparison even though the inclusion periods were not identical. Nonidentical inclusion periods could be confounded by differences in drug supply. However, this confounding factor is probably small because the inclusion periods are close in time and seizure numbers from the police and annual overdose deaths in 2017-2019 did not differ significantly.[2,28]

Although equal cut-off concentrations were set, it is more challenging to interpret postmortem findings than findings from the non-fatal overdose cases because of postmortem redistribution and formation or degradation. The concentrations from the overdose deaths might not be representative of concentrations antemortem.[29] Furthermore, the degree of metabolism and excretion prior to death will be affected by the time from drug administration until death.[29–31] The time period between death and autopsy may also vary, and within this period changes in drug concentrations in the blood samples are likely to take place. Changes in drug concentrations can also be seen in vitro after sample collection from dead and living persons.[32] We have only included analytical results from femoral blood from the overdose deaths to reduce site-dependent postmortem variation.

The conversion factors for blood concentrations of pooled opioids and pooled benzodiazepines are based on a limited number of studies investigating psychoactive effects among opioid-naive individuals.[15] In the present study, we have estimated and compared pooled concentrations in patients who have probably developed tolerance to opioids to different degrees. However, the comparison at group level will not be strongly influenced by the individual differences. Furthermore, the pooled calculations of opioids do not include all common opioids and an underestimation of the results is therefore likely. However, the calculations were equal in both groups, and therefore the underestimation is present in both groups and does not bias the results.

According to the annual number of overdose calls in Oslo, it was expected that approximately two patients would be admitted to the hospital for treatment and inclusion in the study every week, but very

few patients (n = 31) were actually hospitalized. A qualitative study is currently being conducted to better understand why this newly established treatment option was not utilized. All the hospitalized patients who were asked chose to participate in the study. The case-control design used cannot address causation with the limited number of non-fatal overdoses present. A higher number of participants may have resulted in higher ORs and would have allowed for more covariates in the regression analysis. Some of the results of this study have confidence intervals almost overlapping with one, which shows the statistical uncertainty in the analysis. Finding associations for the naloxone results were limited by the low n as well.

We did not adjust for intentional or nonintentional intoxication in this study. Different prevalences of suicide or suicide attempt in the two groups could therefore bias the results.

Drug injection and/or polydrug use are more prevalent in Norway than in countries such as Estonia, Finland and Cyprus.[3,4,33] The results may therefore not be fully generalizable to other countries where injection and polydrug intake is less common.

5 | CONCLUSIONS

Among the fatal overdoses there were higher concentrations of opioids, benzodiazepines and amphetamines. Among the non-fatal overdoses, naloxone was detected in significantly higher concentrations when the pooled opioid concentration increased.

Both groups were polydrug users, indicating that the total load of drug concentration is important to consider, in addition to the number of drugs detected, for a lethal outcome. When determining cause of death, it is important to consider the concentration of all different psychoactive substances. The role of amphetamines in fatal overdoses needs further investigation.

ACKNOWLEDGEMENTS

The study was partly funded by the National Overdose Strategy. The funder was not involved in the planning of the study design or in the collection, analysis or interpretation of data or in the writing of the manuscript. We would like to thank Jelena Staalstrøm and the personnel at the Section for Emergency Addiction Services and Detoxification at the Department on Substance Use Disorder Treatment, OUH, for enabling the study conduction. We would also like to thank Joachim Frost for collecting data from the autopsy cases where forensic toxicological investigations were conducted at St Olav's University Hospital, Trondheim, Håvard Furuhaugen for being responsible for the analysis of the blood samples from the non-fatal overdoses, staff at the Section of Forensic Toxicological Analytics for conducting the toxicological analyses of the autopsy cases and ethanol-analyses of the non-fatal overdoses, and staff at the Section for Forensic Toxicological Support Services for extraction of all analytical results from the LIMS system.

DECLARATION OF INTEREST

None.

AUTHOR CONTRIBUTIONS

H.M.E.E.: conceptualization, formal analysis, methodology, investigation, writing – original draft, visualisation. C.A.: conceptualization, data curation, study implementation and inclusion on site, writing – review and editing. S.T.B.: conceptualization, formal analysis, methodology, writing – review and editing. P.K.: conceptualization, study implementation, writing – review and editing. V.V.: conceptualization, funding acquisition, methodology, writing – review and editing. E.B.R.: conceptualization, data curation, methodology, writing – review and editing, project administration, funding acquisition. All authors read and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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How to cite this article: Edvardsen HME, Aamodt C, Bogstrand ST, Krajci P, Vindenes V, Rognli EB. Concentrations of psychoactive substances in blood samples from non-fatal and fatal opioid overdoses. *Br J Clin Pharmacol*. 2022;88(10):4494-4504. doi:10.1111/bcp.15365