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Development and validation of a liquid chromatography–tandem mass spectrometry (LC–MS/MS) method including 25 novel synthetic opioids in hair and subsequent analysis of a Swiss opioid consumer cohort

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Abstract

Major public health concern is raised by the evidence that common drugs like heroin are now frequently laced or replaced with highly potent novel synthetic opioids (NSOs). The objective of this study was to explore the prevalence and patterns of NSOs in a cohort of Swiss opioid users by hair analysis. Hair analysis is considered an ideal tool for retrospective consumption monitoring. Hair samples from 439 opioid users in Zurich were analyzed. Study inclusion required a previous positive hair test result for heroin metabolites, oxycodone, fentanyl, methadone, or tramadol. The samples were extracted with a two-step extraction procedure, followed by a targeted LC–MS/MS (QTRAP® 6500+) analysis in multiple reaction monitoring mode for a total of 25 NSOs. The method underwent full validation and demonstrated good selectivity and sensitivity with limits of detection (LOD) as low as 0.1 pg/mg. The analyzed sample cohort demonstrated a positivity rate for NSOs of 2.5%, including the following NSOs: butyrylfentanyl, acrylfentanyl, furanylfentanyl, methoxyacetylfentanyl, ocfentanil, U-47700, isobutyrylfentanyl and benzylfentanyl. Furthermore, we were able to identify specific consumption patterns among drug users. The results indicate that hair analysis is a valuable tool for investigating the prevalence of NSOs in drug-using populations, which seems to be low in the case of Swiss opioid users. Nevertheless, the results highlight the need for sensitive analytical detection methods in forensic toxicology to identify and monitor substance distribution in different populations.

KEYWORDS
fentanyl, hair analysis, LC–MS/MS, NSOs, Switzerland

1 | INTRODUCTION

The abuse of opiates such as morphine or heroin has been a global health problem for centuries, but the proliferation of new and highly potent synthetic opioids during the last decade has led to a significant increase in the level of harm.[1] From a chemical perspective, the substances are mainly represented by derivatives of the well-known pain management drug fentanyl, such as acetylfentanyl, butyrylfentanyl, or ocfentanil.[2] Other recent molecular classes that have appeared on the market are the benzamides, the piperazine analogs, the U-series, and the nitazenes with potencies frequently exceeding that of morphine by over 100 times.[3] A major public health concern is raised by the evidence that common drugs like heroin or counterfeit Oxycontin® or Xanax® are now frequently laced or replaced with designer opioids and consequently being consumed by unsuspecting users, regularly leading to overdose fatalities.[4] Recent fatality cases with designer opioid intoxications in the cantons of Zurich and Ticino show that this problem has also been encountered in Switzerland.[5–7]

A comparison with the database of the Zurich Forensic Science Institute (FOR) regarding new psychoactive substances (NPS) and especially NSOs, seized in recent years, showed that the above-mentioned cases probably only represent the tip of the distribution of these substances in the Swiss drug scene. As listed in Table 1, in the last 10 years, 16 NSOs were analyzed and identified by the FOR. The prevalence of NSOs in Switzerland is also displayed by the testing results of the Drug Information Center Zurich (DIZ), which offers free drug checkings as part of its prevention programs.[8] In the year 2021, NPS were detected in 171 cases out of approx. 2500 analyzed samples, including one case with the NSO etonitazepipne. These indications point toward an increased prevalence and, in particular, consumption of NSOs and demonstrate the need for further analytical investigations with regard to this substance group in Switzerland. The main objective of the present study therefore represents the determination of NSO prevalence and consumption patterns among a cohort of Swiss opioid users by means of hair analysis. Hair analysis has become increasingly more important for the analysis of NSOs in forensic toxicology.[9] The keratinized hair matrix is particularly well suited for such prevalence studies because it offers a much wider diagnostic window than, for example, blood or urine.[10] For targeted screening of the typically low-concentrated analytes in hair, liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) in multiple reaction monitoring mode (MRM) will be used. The system combination is currently considered the gold standard for routine high-sensitivity detection and quantification of drug analytes.[11] Subordinate objectives of the present work are therefore the development and validation of an applicable LC–MS/MS method. Thereby the choice of NSOs to be included in the method is primarily based on the aforementioned data of the FOR and the DIZ and on the latest open public NSO prevalence data originating from substance monitoring agencies such as the United Nations Office on Drugs and Crime (UNODC) or European Monitoring Centre for Drugs and Drug Addiction (EMCDDA).

TABLE 1 Seized novel synthetic opioids from the Zurich Forensic Science Institute from the years 2013 to 2023.

Seized opioids
4-ANPP
4-Fluorisobutyrylfentanyl
Acetylfentanyl
Acrylfentanyl
AH-7921
Benzylfentanyl
Butanoylfentanyl
Cyclopropylfentanyl
Fentanyl
Furanylfentanyl
MT-45
U-47700
W-15
Isotonitazene
Ocfentanil
Metonitazene

2 | EXPERIMENTAL

2.1 | Chemicals and reagents

The reference standards: remifentanil, ocfentanil, benzylfentanyl, α-methylfentanyl, cyclopentylfentanyl, cyclopropylfentanyl, 4-fluoroisobutyrylfentanyl, methoxyacetylfentanyl, 4-chloroisobutyrylfentanyl, U-49900, AH-7921, acetylfentanyl, U-51754, norfentanyl, 4-ANPP (N-phenyl-1-[2-phenylethyl]-4-piperidinamine), acrylfentanyl, alfentanil, butyrylfentanyl, fentanyl, furanylfentanyl, sufentanil, U-47700, acetylfentanyl-d5, and fentanyl-d5 were purchased from Cerilliant (Round Rock, TX). The opioid reference standards, namely, isobutyrylfentanyl, MT-45, and valerylfentanyl, were purchased from Cayman Chemical (Ann Arbor, MI). The standards were diluted with LC–MS grade methanol (Chromasolv®), purchased from Sigma-Aldrich (Buchs SG, Switzerland), to appropriate working solutions and stored at −20°C until use. For the LC–MS/MS mobile phases, LC–MS grade acetonitrile purchased from ACROS ORGANICS (Fisher Scientific AG, Switzerland), LC–MS grade water (Chromasolv®) from Sigma-Aldrich and ammonium formate and formic acid obtained from Merck (Darmstadt, Germany) were used.

2.2 | Hair matrices and sample preparation

Blank hair used for validation originated from volunteers that gave oral consent. The blank hair pools were negatively tested for the most common illegal street drugs from various substance classes using the multi-analyte approach by Scholz et al.[12] The screened authentic hair

samples ($n = 439$) originated from routine casework conducted at the Zurich Institute of Forensic Medicine, Center for Forensic Hair Analytics (Zurich, Switzerland), between the years 2015 and 2022. This routine casework typically includes driving ability examinations or monitoring of substitution programs as well as criminal cases. Cohort inclusion required a previous positive hair testing result for one or more of the following opioids: oxycodone, fentanyl, methadone, tramadol, heroin (6-monoacetylmorphine), and morphine. The positivity rates of the samples for the individual opioids were as follows: oxycodone = 39%, fentanyl = 44%, methadone = 27%, tramadol = 26%, heroin (6-monoacetylmorphine) = 39%, and morphine = 45%. The analyzed hair length was in general 5 cm. In certain cases, the hair strands were segmented into two individual segments of 2.5 cm and then analyzed individually (see also Table 2). Hair samples, both the ones that originated from blank hair pools for the validation (calibrators, quality controls [QC] and blanks) and the authentic hair samples from routine casework, were extracted in accordance with the procedure described by Scholz et al.[12,13] In brief, the main steps of the extraction protocol included a consecutive washing process with water, acetone, and hexane followed by cutting the hairs into snippets and pulverization. A two-stage extraction was then performed using methanol and a buffered methanol–water mixture for 90 min each. The extracts were then evaporated, resuspended in eluent A, and measured on the LC–MS/MS system. Hair samples from cases that were included in the cohort with a sampling date prior to the method validation were all extracted according to the described procedure and analyzed in one batch. After method validation, hair sample extracts from routine casework were screened when they fulfilled the study inclusion criteria. The storage time of sample extracts was less than 12 months at −20°.

2.3 | Working solutions, calibrators, and quality controls

For the preparation of the calibrator and QC samples, methanol-based working solutions containing all NSOs at concentrations of 0.2, 2, 20, and 200 pg/µL were prepared. Furthermore, an internal standard working solution containing both the deuterated opioids acetylfentanyl-d5 and fentanyl-d5 was prepared at a concentration of

TABLE 2 Comprehensive listing of cases tested positive for NSOs, along with the corresponding identified analytes and their respective concentration values.

Case Nr.	Sampling date (month/year)	Drugs present in the sample	Detected NSOs (pg/mg hair)
1.	07/2016	Morphine, heroin, codeine, fentanyl, tramadol, methadone, cocaine, methylphenidate	Butyrylfentanyl (18), isobutyrylfentanyl (20)
2.	01/2018	Oxycodone, oxymorphone, fentanyl, methylphenidate	Methoxyacetylfentanyl (10)
3.	10/2018	Morphine, hydromorphone, acetylcodeine, codeine, hydrocodone, dihydrocodeine, oxycodone, oxymorphone, fentanyl, tramadol, methadone, cocaine, amphetamine, methamphetamine, ketamine, tizanidine, alproazolam, clonazepam, diazepam, oxazepam, midazolam	Ocfentanil (9.3/8.0), furanylfentanyl (22/19)
4.	03/2019	Fentanyl	Acrylfentanyl (<LLOQ)
5.	12/2021	Codeine, hydrocodone, fentanyl, methadone	Acrylfentanyl (0.12)
6.	02/2022	Morphine, heroin, hydromorphone, codeine, fentanyl, methadone	Benzylfentanyl (0.40)
7.	12/2019	Cocaine, amphetamine, methamphetamine, methylphenidate, ketamine, morphine, heroin, codeine, oxycodone, methadone	Butyrylfentanyl (<LLOQ), U-47700 (0.10)
8.	07/2020	Cocaine, amphetamine, methamphetamine, methylphenidate, ketamine, morphine, heroin, codeine, oxycodone, methadone	Butyrylfentanyl (<LLOQ), U-47700 (0.11)
9.	12/2020	Cocaine, amphetamine, methamphetamine, methylphenidate, ketamine, morphine, heroin, codeine, oxycodone, methadone	Butyrylfentanyl (<LLOQ), U-47700 (0.19)
10.	07/2021	Cocaine, amphetamine, methamphetamine, methylphenidate, ketamine, morphine, heroin, codeine, oxycodone, methadone	Butyrylfentanyl (<LLOQ)
11.	10/2022	Cocaine, amphetamine, methamphetamine, methylphenidate, ketamine, morphine, heroin, codeine, oxycodone, methadone	Butyrylfentanyl (0.56), U-47700 (1.65)

Note: In case 3, two hair segments were available and the measured concentrations are separated by a slash ("/"). Furthermore, the table provides information on the sampling time points and other detected classical drugs.

40 pg/µL in methanol. Both working solutions were stored at −20°C in amber vials until the next preparation step. Calibrators were prepared at concentrations of 0.1, 0.5, 1, 5, 10, 50, 100, 500, and 1000 pg/mg of hair, and QCs at 1.2, 75, and 800 pg/mg of hair by spiking the blank samples accordingly.

2.4 | LC–MS/MS conditions

The chromatographic separation of the analytes was achieved by a Prominence UFLC system (Shimadzu, Kyoto, Japan) equipped with a Kinetex XB-C18 (2.6 µm, 50 × 2.10 mm) column, maintained at 40°C. The samples were stored in the autosampler at 15°C, and the injection volume was 10 µL in all cases. Elution was performed by a gradient, using 20 mM ammonium formate with 0.1% formic acid in water as eluent A and 20 mM ammonium formate with 0.1% formic acid in acetonitrile as eluent B. The initial flow rate was 0.6 mL/min and was increased to 0.75 mL/min at 3 min. The gradient settings were as follows: 0.01 to 1.5 min with 5% eluent B; 1.5 to 4 min with 15% eluent B; 4 to 9 min increasing to 25% eluent B; 9 to 10 min to finally 95% eluent B; and 11 to 11.01 min decreasing to starting conditions (5% eluent B), which were maintained until the end of the run at 12 min. The detector, a QTRAP® 6500+ linear ion trap mass spectrometer (Sciex, Darmstadt, Germany), was operated in electron spray ionization (ESI) positive mode with an ion spray voltage of +4500 V. Multiple reaction monitoring mode was used on the MS, and various parameters such as collision energy (CE), entrance potential (EP), collision cell exit potential (CXP), and declustering potential (DP) were optimized for each analyte. MS parameters are listed in Table S1. Each analyte was identified by one precursor ion (Q1) and two characteristic product ions (Q3). To screen and confirm the presence of classical opioids in the cohort samples, transitions of certain classical opioids were also included in the MS/MS method. The retention time tolerance was set to 20 s, and the target scan time was 1.89 s. Nitrogen was used as the curtain gas and was fixed at 20 psi. The collision gas was set to medium, the ion source gas 1 was set to 70 psi, and the ion source gas 2 was set to 50 psi. The source temperature was maintained at 450°C.

2.5 | Method validation

Ensuring qualitative and quantitative comparability of the results, the developed method was validated in accordance with the guidelines of the GTFCh (Society of Toxicological and Forensic Chemistry).[14,15] The validation took place in terms of selectivity, linearity, accuracy, precision (intra- and inter-day precision and accuracy), limit of detection (LOD) and lower limit of quantification (LLOQ), specificity (including matrix effect), recovery (extraction efficiency) and stability. For the purpose of normalization, the samples were spiked with 2000 pg of each of the internal standards acetylfentanyl-d5 and fentanyl-d5 during the validation procedure. During the analysis of authentic hair samples processed between the years 2015 and 2022, exclusively, fentanyl-d5 (2000 pg) was employed as an internal standard. As a result, fentanyl-d5 was solely used for the normalization of the results within the authentic hair sample cohort.

Since the hair sample extracts from the cohort were stored for up to 1 year at −20° until measurement, the stability of the NSOs potentially present in the extracts was evaluated. Therefore, fentanyl was measured in extracts of three QC samples (with concentrations of 2, 25, and 250 pg/mg, respectively) and in a hair pool (positive for fentanyl, approximately 6.25 pg/mg) over 12 months. The samples originated from the months between October 2022 and October 2023 from corresponding routine casework measurement series.

2.6 | Data analysis

Quantification was achieved using SCIEX OS (version 2.0.1.48629 SCIEX, Darmstadt, Germany) and Analyst™ software (version 1.7.3, SCIEX, Darmstadt, Germany).

3 | RESULTS AND DISCUSSION

By optimizing the eluent gradient and the flow rate, an LC-method was obtained that was capable of adequately separating all 25 NSOs including the isomers butyrylfentanyl (7.24 min) and isobutyrylfentanyl (7.03 min) (Figure 1). The total run time, including the time needed to re-equilibrate the column before the next injection, was 13 min. Table S1 lists the retention times of all analytes. Retention times ranged from 2.84 min (norfentanyl) to 9.14 min (cyclopentylfentanyl). Figure 1 shows the chromatogram recorded from a blank hair sample spiked with all analytes at a concentration of 20pg/mg.

To the best of our knowledge, only few LC–MS/MS-based targeted hair analysis methods focusing on the analysis of NSO have been published so far.[11,16–20] With a total of 25 NSOs our method represents a valuable addition to the knowledge pool in the field.

3.1 | Validation

Validation settings are summarized in Tables S2, S3 and S4. The chromatograms of constituents of drug-free human hair ($n = 6$) showed no interfering signals at the corresponding retention times. Calibration curves were linear ($R^2 > 0.99$) within their concentration ranges (see Supporting Information, Table S2). For the analytes MT-45, norfentanyl, ocfentanil, α-methylfentanyl, 4-chloroisobutyrylfentanyl, U-49900, and AH-7921, a quadratic model ($1/x^2$) was used to compensate for the heteroskedastic distribution of the values of the calibration curve. In contrast, for all the other analytes, a linear weighted (1/x) model was used, which was considered more suitable for calculating the calibration curves. LOD and LLOQ values were calculated and determined by applying signal-to-noise (S/N) ratios of 3:1 and 10:1, respectively. For most analytes, the LOD was 0.1 pg/mg most (detailed values can be obtained from Table S2). The sensitivity of the method is comparable with other LC–MS/MS-based hair analysis

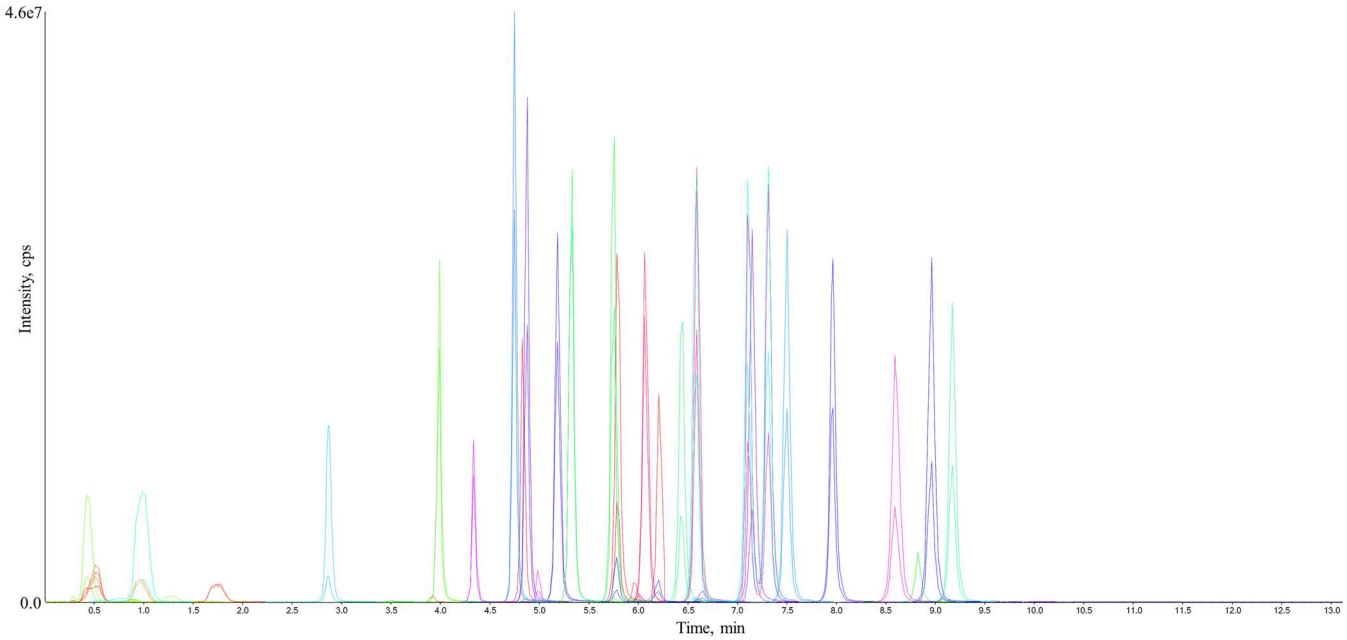

FIGURE 1 LC–MS/MS chromatogram of a blank hair sample, spiked with 20 pg/mg hair reference standard of each analyte included in the method.

methods published.[16–18,21] For most of the analytes at all three QC concentration levels (1.2, 75 and 800 pg/mg), bias and imprecision data were within the GTFCh criteria (bias ± 20%, imprecision ± 15%). Matrix effects were mostly insignificant (<±30%), and recoveries were acceptable for all compounds. Individual parameters of the few analytes that did not meet the acceptance criteria can be found in Tables S3 and S4. The quantification for these analytes may not be completely accurate, but the method can still be used to identify these substances. The NSOs detected in the hair extracts (see chapter below) were not affected by deviating parameters, and the quantification of these analytes can therefore be considered valid.

As described in the experimental section, the stability of NSOs in hair sample extracts was assessed by analyzing different QC samples and real hair pool extracts for their fentanyl concentration over 12 months. The fentanyl concentrations measured in the 12 months samples were correlated, and the average percentual decrease was calculated using the linear equation. In no case, the average decrease was higher than 20% (see Figure S1). The stability of fentanyl in hair sample extracts stored at −20° met the acceptance criteria. However, the stability data for fentanyl cannot be transferred 1:1 to the other NSOs, especially to those that differ structurally from fentanyl. Therefore, it cannot be excluded that some positive cases, extracted in the past, have been overlooked. To our knowledge, further stability data on NSO containing hair extracts are not available in literature.

3.2 | Authentic hair samples: detected NSOs

A total of 439 hair sample extracts was successfully analyzed using the developed method. The investigated cohort showed positive testing results for NSOs in 11 different cases, corresponding to a positivity rate of 2.5%. The evaluated value is similar to other European studies on the prevalence of NSOs in hair, such as the ones published from Larabi et al.[11] with 2.2% or from Freni et al. with 3.4%.[18] The identified NSOs in our cohort were ocfentanil, furanylfentanyl, acrylfentanyl, methoxyacetylfentanyl, butyrylfentanyl, benzylfentanyl, isobutyrylfentanyl, and U-47700. With the exception of U-47700, which can be attributed to the molecular class of the benzamides, the detected NSOs exclusively consisted of fentanyl derivatives (fentalogs). From a legal point of view, all substances are listed in the narcotics directory in Switzerland, thus making possession, distribution, or consumption a legal offense.[22] The fentalogs acrylfentanyl, benzylfentanyl, and isobutyrylfentanyl were, to the best of our knowledge, the first time ever detected in authentic human hair samples.

As illustrated in Table 2, acrylfentanyl was detected in two cases (4 and 5). Concentration in case 4 was below LLOQ and in case 5 was 0.12 pg/mg hair. The interpretation of such low hair concentrations with regard to the nature of consumption is generally difficult. Here, the single or occasional exposure to the substance could either be on purpose or unconsciously as of adulteration of the other drugs (fentanyl, codeine, hydrocodone, etc.) that were consumed in the two cases. Acrylfentanyl has been a relatively widespread NSO with a potency similar to that of fentanyl and has been responsible for dozens of overdose fatalities in Europe.[23] In Sweden, for instance, over 40 people died from the substance in 2016 within a few months.[24]

Benzylfentanyl, a relatively rarely reported NSO with a presumably low potency of about half that of morphine, was detected in case 6 from the year 2022. Despite the relatively low potency of the substance, there have been reports on overdose fatalities in the past.[25] Although the detected concentration in the present case is very low

(0.4 pg/mg hair), it demonstrates that classical fentanyl derivatives still appear to be in circulation in recent times. As evident from Table 2, the patient from case 6 is a classic opioid user who tested positive for various traditional opioids such as morphine, heroin, hydromorphone, and codeine.

In the hair sample of case 1, isobutyrylfentanyl was found at a concentration of 20 pg/mg hair. With regard to the relatively high potency of approximately 25 times that of morphine, the measured concentration probably reflects occasional or single exposure to the substance. Because the patient showed a multivariant drug consumption behavior, involving different classical opioids as well as stimulants (cocaine and methylphenidate) and furthermore was positive for a second NSO (butyrylfentanyl) at a similar concentration (18 pg/mg), it is well possible that, in this case, the NSOs were consumed purposely on a recreational base.

Although having a similar potency, in contrast to isobutyrylfentanyl, its isomer butyrylfentanyl was involved in several overdose fatalities globally and also in Switzerland.[7,26] As evident from Table 2, butyrylfentanyl was also detected in cases 7–11, thereby being the most frequently detected NSO in the examined sample cohort. In four of these five cases, alongside butyrylfentanyl, the benzamide NSO U-47700 was simultaneously detected. The measured concentrations in these samples were consistently within a similar, low picogram range. This observation raised suspicion that the hair samples originated from a single cohort member who underwent multiple hair sample submissions for analysis over a span of approximately 3 years. Further evidence supporting this hypothesis of a long-term NSO consumer was that, alongside the mentioned NSOs, particularly identical traditional drugs, including various amphetamines and a substantial quantity of classical opioids, were present in the hair samples. Moreover, the sampling times followed regular intervals of 6 months, respectively 1 year. Such intervals align with those commonly observed in abstinence or substitution therapy control programs. Consequently, it is highly likely that the analyzed hair samples were obtained from a single individual that used the aforementioned NSOs over an extended period. However, because of the anonymization of the cohort, it was not possible to definitively confirm this suspicion.

The results from case 3 also indicate intentional NSO intake. In contrast to the other cases, two hair segments of 2.5 cm length were available in case 3. Each segment reflected the consumption pattern of 2–3 months. The two hair segments were positive for the NSOs ocfentanil and furanylfentanyl, which both have a relatively high potency, about 100 times that of morphine, and have caused occasional fatalities.[27–31] The measured concentrations were 9.3/8.0 pg/mg for ocfentanil and 22/19 pg/mg for furanylfentanyl, respectively, and were thus in the upper range of the measured NSO values of our positive cases. Similar to case 1, the multivariant consumption behavior observed in case 3 indicates that the NSOs might have been consumed intentionally. The exposure to NSOs poses high risks to users not only because of their potency and the resulting possibility of death from overdose but also because of their unknown long-term side effect profile. In vitro mammalian cell micronucleus tests on ocfentanil, furanylfentanyl, and acrylfentanyl revealed genotoxicity raising severe concern of long-term consequences for suspected or unsuspected consumption.[32]

Methoxyacetylfentanyl with a potency of approx. 20 times that of morphine was involved in 13 overdose fatalities in Europe between the years 2016 to 2018.[33,34] Within this time frame, also the hair sample from case 2 was obtained (2018) and tested positive for methoxyacetylfentanyl at a concentration of 10 pg/mg hair. In Europe, methoxyacetylfentanyl was mainly sold on the surface web in the form of powder or "ready to use" vape or nasal spray liquid and disappeared from the market within the year 2019 rapidly.[34] However, overdose fatality case reports from the United States from the year 2021 prove that on the global level, the substance did not entirely disappear from the market.[35]

Overall, the results of the study demonstrate that the hereindeveloped hair analysis method is a valuable tool to monitor the prevalence, as well as trends and changes, in the consumption habits of Swiss drug and opioid users with regard to NSOs. For the first time, it was possible to obtain evidence for the presence and consumption of methoxyacetylfentanyl and isobutyrylfentanyl in Switzerland that had not previously been reported by institutes such as the FOR or the DIZ. The results of the study further indicate that the adaptation of narcotics laws in Switzerland and the associated ban of all NSOs found in this study did not prevent their use. Although the prevalence of NSOs among Swiss opioid users seems to be relatively low, it appears to be reasonable to include the developed method in routine analysis, especially in suspected cases (multivariant drug consumption behavior). The method should also be updated with respect to newer emerged NSOs like the nitazenes, which unfortunately were not available as reference standards at the time of validation (2020). According to our study results, the fentanyl derivatives are still detectable in hair sample extracts stored at −20° for up to 1 year and proved to be relatively stable under these conditions. However, in order to exclude any potential degradation of NSOs over time, analysis using the developed method will in future be performed on an ongoing basis.

4 | CONCLUSION

This study presents a fully validated LC–MS/MS-based hair analysis method for the targeted analysis of 25 NSOs. The subsequent prevalence study among Swiss opioid users revealed a positivity rate for NSOs of 2.5%, including the detection of acrylfentanyl, benzylfentanyl, and isobutyrylfentanyl, which were detected for the first time ever in authentic human hair samples. Based on these findings, it can be concluded that the use of the described method in forensic and toxicological routine analysis represents a powerful tool to monitor the prevalence and consumption patterns of NSOs in specific populations. This ultimately aims at case-specific decision finding in routine analysis and helps to develop appropriate public health and policy interventions to mitigate the risks associated with NSO abuse. Further surveillance and research are needed to stay ahead of emerging NSOs and to address the evolving landscape of substance abuse.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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