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Reviews

Ten Years of Fentanyl-like Drugs: a Technical-analytical Review

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Synthetic opioids, such as fentanyl and its analogues, are a new public health warning. Clandestine laboratories produce drug analogues at a faster rate than these compounds can be controlled or scheduled by drug agencies. Detection requires specific testing and clinicians may be confronted with a sequence of severe issues concerning the diagnosis and management of these contemporary opioid overdoses. This paper deals with methods for biological sample treatment, as well as the methodologies of analysis that have been reported, in the last decade, in the field of fentanyl-like compounds. From this analysis, it emerges that the gold standard for the identification and quantification of 4-anilinopiperidines is LC-MS/MS, coupled with liquid-liquid or solid-phase extraction. In the end, the return to the scene of illicit fentanyls can be considered as a critical problem that can be tackled only with a global multidisciplinary approach.

Keywords Fentanyl, designer opioids, fentanyl-like compounds, illicit fentanyls, mass spectrometry

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1 Introduction

Fentanyl is a potent μ-opioid agonist, which has been used therapeutically, owing to its high lipid solubility and potency (50 – 100 fold higher than morphine), as both an analgesic and anaesthesia adjuvant. Fentanyl and its derivatives (Fig. 1) such as sufentanil, alfentanil, remifentanil and carfentanil, belong to the class of 4-anilinopiperidines and exert their pharmacological action through interaction with the μ-opioid receptor.[1] Their adverse effects are related to dose, which include respiratory depression, sedation, nausea, vomiting, constipation, pruritus, physical dependence, the risk of addiction, bradycardia, and skeletal muscle rigidity.[2,3] Immediately after their introduction into the market as drugs for the treatment of pain, there was a significant increase in abuse, off-label and illicit uses.[4-6] Besides a series of illicit fentanyls (Fig. 2), also known as non-pharmaceutical fentanyls or designer fentanyls, have been developed, while causing a major health risks problem.[7-9] The first far-reaching illicit use of fentanyl analogues (identified

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Francesca Faggiani obtained the degree in Pharmacy in 2018 at the University of Milan. During her university studies she developed a strong interest for analytical chemistry applied to forensic toxicology that she has expanded studying and writing her thesis. She is now involved in a start-up project for a multinational corporation operating in the pharmaceutical field.

Marco Pallavicini received his PhD in "Chimica del Farmaco" in 1986 at Milan University. In 1990, he became Assistant Professor at the same university and, eight years later, Associate Professor. Since 2005, he has been Full Professor of Medicinal Chemistry at Milan University. From medicinal chemistry, his interest has extended to analytical characterization of biological active compounds and their precursors in unichiral form, in particular by NMR spectroscopy, DSC and chiral HPLC.

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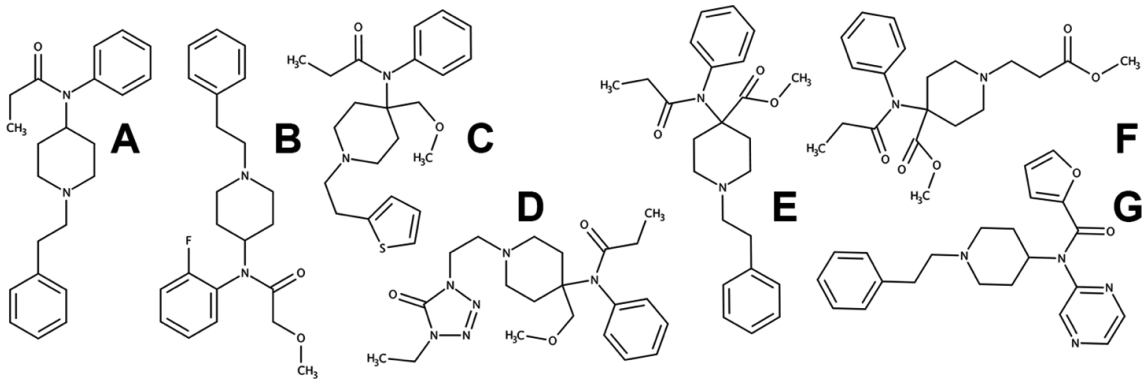

Fig. 1 Chemical structures of some clinical opioids: fentanyl (A), ocfentanil (B), sufentanil (C), alfentanil (D), carfentanil (E), remifentanil (F) and mirfentanil (G).

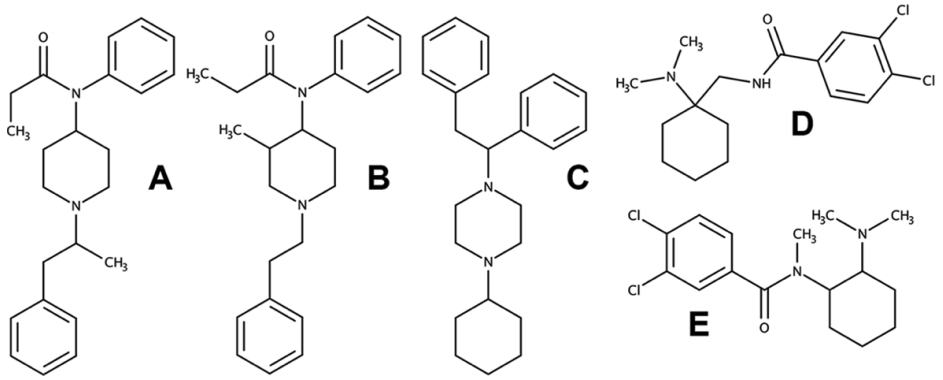

Fig. 2 Chemical structures of common illicit fentanyls: alpha-methylfentanyl (A), 3-methylfentanyl or mefentanyl (B), MT-45 (C), AH-7921 (D) and U-47700 (E).

with the street names of China White or Synthetic Heroin) was in California between 1979 and 1988.[10] The composition of China White is complex and non-standardizable. Scientific literature reported a mixture of *p*-fluorofentanyl, alpha-methylfentanyl, 3-methylfentanyl, acetyl-fentanyl which may include heroin constituents.[11–14] The danger related to fentanyl derivatives is due to: 1) the variability of compounds that may exist in a given sample 2) the inconsistency of dosages 3) their potency 4) easy access by consumers through the deep web or black market[14,15] and 5) fatal respiratory depression. In recent years, the great spread of fentanyl-like compounds has created massive problems from social, health, normative and analytical points of view. Hundreds of analytically confirmed deaths have been from synthetic opioids-related problems within the past years. They are rising at a worrying rate while confronting those produced by the misuse of natural and semi-synthetic opiates.[16]

In this paper, we focus on the problem of identifying and quantifying these substances, which is always very difficult because of the low dose administered and their chemical heterogeneity. Thus, the instruments must be sensitive enough to detect them, and be equipped with high resolution for the identification of unknown molecules.[17] The class of illicit fentanyls includes a long list of fentanyl derivatives, such as: 1) methyl-analogues, 2) W-series, 3) acetyl-analogues, 4) butyr-analogues, 5) thio-analogues, 6) hydroxy-analogues, 7) furanyl-analogues, 8) benzyl-analogues, 9) cyclo-analogues, 10) acrylfentanyl, 11) ocfentanil, 12) fluoro-analogues and 13) other chemically unrelated opioid agonists. Among the latter significant examples are piperazine derivatives (such as MT-45) and benzamide ones (such as AH-7921, U-47700 and U-50488).

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Michele Dei Cas obtained the Degree in Pharmacy in 2017 at the University of Milan. Since 2017, he is a PhD student at the Doctorate School in Molecular and Translational Medicine at the same university. His scientific interests are related to the application of advanced analytical techniques in the fields of forensic toxicology, metabolomics, and clinical biochemistry.

Table 1 Extraction procedures used in different matrices for the isolation of fentanyl-like compounds; "screening" means a method comprehensive of multiple fentanyl-like drugs

Extraction	Fentanyl analogues	pH samples	Matrix	References
Acid hydrolysis + single-phase extraction	Screening	4	Hair	35
Acid hydrolysis + single-phase extraction	Fentanyl	4 - 6	Bone and bone marrow	36
Automated on-line SPE[a]	Screening	—	Dried blood spot	68
Back extraction	Carfentanil	11	Blood	69
dLLME[b]	Screening	9	Plasma, urine	45
Enzymatic hydrolisis + LLE[c]	Screening	9	Urine, blood	38, 39
Enzymatic hydrolysis + SPE	Screening	5	Urine	1, 28
Enzymatic hydrolysis + SPE	U-47700	3 - 5	Urine	32
HF-LPME[d]	Screening	10	Biological samples	44, 45
LLE	Butyrfentanyl	12	Blood, liver, urine, gastric content and vitreous	70
LLE	Furanyl fentanyl	11	Femoral blood	71
LLE	Ocfentanil	10	Tissues and seized drug	72
LLE	Screening	10 - 12	Blood, plasma, urine, oral fluid	17, 57, 73 - 75
LLE	Screening	—	Plasma	76
LLE/SPE/dLLME	Fentanyl	11	Urine	47
LLE + acid back-extraction	Acetyl fentanyl	11	Blood, liver, brain and urine	40
LLE + back extraction	Screening	8.2	Blood, urine, vitreous	41
MEPS[e]	Remifentanil	5	Plasma	48
On-line SPE	Screening	9.3	Plasma, urine	77, 78
PP[f]	Carfentanil	—	Blood	79, 80
PP	Remifentanil	5	Blood and plasma	81
PP	Screening	—	Blood, serum, plasma, DBS	42, 43, 82
PP + hSPE[g]	*o*-Fluorofentanyl	—	Serum, blood and urine	83
PP + LLE	Remifentanil	11	Plasma	84
PP + LLE	Screening	10	Plasma	37
PP + SPE	Screening	6 - 7	Blood	27
SA-PEME[h]	Screening	4	Plasma, urine, breast milk	46
Single-phase extraction	Fentanyl	—	Saliva and plasma	34
Single-phase extraction	Screening	4	DBS	33
SPE	Acrylfentanyl	6 - 7	Blood	85
SPE	Butyrfentanyl	8 - 9	Post-mortem fluids and tissue	86, 87
SPE	Fentanyl	8.8	Plasma	88
SPE	Screening	5 - 6	Blood, serum, tissue, urine	26, 30, 31, 53 - 55, 89, 90
SPE	Screening	9	Urine	78
SPE	Screening	6	Blood, vitreous, bile, gastric content, urine, brain, meconium and liver tissues	91, 92
SPE	Sufentanil	3	Plasma	93 - 95
SPE cation exchange	Screening	5	Urine	96
SPE mixed mode	Alfentanil	5	Plasma	97

a. SPE, Solid phase extraction. b. dLLME, Dispersive liquid-liquid microextraction. c. LLE, Liquid-liquid extraction. d. HF-LPME, Hollow fiber assisted liquid-phase micro extraction. e. MEPS, Microextraction in packed syringe. f. PP, Protein precipitaion. g. hSPE, Hybrid solid phase extraction. h. SA-PEME, Surfactant assisted pulsed two-phase electro membrane extraction.

2 Methods

A literature search was performed on the Pubmed database and several governmental and institutional websites, while taking into consideration any analytical methods of detection, extraction and determination of fentanyl, fentanyl analogues, illicit fentanyl or designer fentanyls with particular focus on LC-MS and GC-MS. The following sequence of keywords was used: (fentanyl OR illicit fentanyl OR names of particular designer fentanyls) AND (analytical OR extraction OR detection OR method OR determination OR analysis OR LC-MS OR GC-MS). In this review we have included only those articles that are more recent than ten years and had abstracts available in the English language. All articles were then selected to determine their relevance within the framework of the present review.

3 Analysis of Fentanyl-like Compounds

3·1 Extraction techniques

The techniques that are most widely used to extract fentanyl-like compounds from complex/biological matrices are solid-phase extraction (SPE) and liquid–liquid extraction (LLE). SPE[18-20] and LLE[21-23] are reliable and widespread techniques used in the field of chemical-toxicological analysis for detecting of the majority of toxic compounds. Several slightly different protocols for fentanyls extraction were evaluated throughout the literature. In the main text, the most significant methodologies will be discussed, while a concise overview of variations and alternative methods can be found in Table 1.

SPE is currently the most widely used sample-preparation technique used for chemical analysis in different areas of interest.[24,25] The extraction process is based on the interaction of the analytes of interest, dissolved in a liquid phase, with an adsorbent-solid phase. SPE is an attractive replacement for LLE, since it has some advantages. Compared to LLE, the SPE allows to: 1) considerably reduce the consumption of solvents, 2) obtain higher recovery factor, 3) achieve highly purified extracts, and 4) can be extremely selective since it is possible to choose between a wide range of adsorbents and solvents. SPE-screening methods are commonly performed under acidic conditions (pH < 6) providing protonation of the amine group of the analytes. Moreover, the absorbent of cartridges can be either reverse-phase,[26] cation exchange,[27,28] polymeric[29] or mixed-mode.[30,31] In specific cases, further steps were implemented prior SPE, namely solvent protein precipitation[27] or enzymatic hydrolysis on urine samples.[1,28,32]

According to Eckart *et al.*[26] serum, blood and tissue samples were purified for the detection of different opioids using a

Bakerbond SPE C18. Plasma was diluted with phosphate buffer (pH 6), added in an internal standard, centrifuged and applied to SPE. Otherwise, postmortem tissues were homogenized in a 0.9% saline solution and an aliquot applied to SPE. After washing, the alkaline analytes were eluted with dichloromethane/isopropanol/ammonium hydroxide (40:10:2). They were then evaporated and reconstituted with acetonitrile/methanol/water (3:3:2).

LLE is the traditional method in which analytes, contained in a sample are separated based on their solubility in two different immiscible liquid solvents. Generally, the efficiency of an LLE process can be strongly improved by modifying the distribution coefficient: acid and basic compounds would prefer non-polar solvents at low (pH < 6) and high pH (pH > 8) respectively. LLE-screening methods are generally performed under basic conditions (pH > 8 – 9), providing deprotonation of the amine of the analytes. The extraction solvents, used on their own or in a mixture, are diethyl ether, ethyl acetate, hexane, toluene, isoamyl alcohol, acetonitrile, acetone, ter-butyl methyl ether and butyl acetate. In some cases, single-phase extraction was made using a water-miscible mixture of solvents, particularly, methanol and acetonitrile.[33–36] Further steps can be implemented coupled with LLE, such as solvent protein precipitation,[37] enzymatic hydrolysis on urine samples[38,39] or back-extraction.[40,41]

Caspar *et al.*[17] applied a LLE on blood diluted with a saturated aqueous sodium sulfate solution, and then extracted with a diethyl ether–ethyl acetate mixture (1:1). The mixture was shaken, centrifuged and the upper organic extract was transferred. Sodium hydroxide and diethyl ether–ethyl acetate mixture were added to the remaining liquid, and again mixed, and centrifuged. The upper solvent phase was transferred and evaporated. The analytes resolved in methanol +0.1% formic acid (FA) in water.

Protein precipitation (PP) is used to eliminate protein-contaminants in samples. It can be used on its own or coupled with other extraction techniques. Methods to precipitate proteins are salting out, isoelectric precipitation, precipitation with miscible solvent, polyvalent metallic ions and others. In order to easily recover fentanyl-like compounds, precipitation with acetonitrile, methanol and ethanol was carried out.[42,43] Simplicity and rapidity are the main advantages of this kind of method, thus making it appealing.

Rarely, other microextraction procedures were employed, such as hollow fiber-assisted liquid-phase micro extraction[44,45] (HF-LPME), surfactant-assisted pulsed two-phase electro membrane extraction[46] (SA-PEME), dispersive liquid–liquid microextraction[45,47] (dLLME) and microextraction in a packed syringe[48] (MEPS). MEPS[48] employed a low volume of samples, reaching low limits of quantification. dLLME[45,47] is a straightforward and low-cost technique. It is frequently employed for simple samples, such as tap and river water, but not recommended for the extraction of biological samples. By contrast, HF-LPME[44,45] has different disadvantages, such as the formation of air bubbles, time-consuming extraction, low precision, the high cost of extraction fibers, which are also fragile and have a limited lifetime. All of those new microextraction methods represent a viable development of the traditional LLE and SPE, although they remain the second choice.

The use of stable-isotope labelled as an internal standard improves the quali-quantitative performances, since they share with the analyte most of the biological and physiochemical properties. Examples of commercially available isotopically labelled internal standard are fentanyl-D_5, acetyl fentanyl-$^{13}C_6$, acetyl norfentanyl-$^{13}C_6$, norfentanyl-D_5 and sufentanil-D_5.

3·2 Analytical techniques

As for extraction paragraph, in the main text the most significant methodologies of analysis will be discussed, while a concise overview of the variations and alternative methods can be found in Table 2.

Highly sensitive analytical methods are required for the detection of fentanyl-like compounds, since they are very potent, short-acting opioids and occur in low concentrations. LC-MS/MS is indeed the more popular analytical technique in the field of bioanalysis,[49–51] and also for fentanyl-like compounds. Several papers reported a similar LC-MS/MS method: many of them share 1) the C18-chromatographic column, 2) a gradient elution program, 3) an ESI source operating in the positive ionization mode, 4) a targeted multiple reaction monitoring (MRM) scan mode and 5) the same combination of mobile phases, commonly acetonitrile or methanol coupled with water or buffer. The use of LC-MS compatible ammonium counter-ions (formate, acetate or hydroxide) in the mobile phase has been implemented in different studies, but its use seems not to be mandatory, since negatively charged moieties are not present in fentanyls.

Fentanyls form stable protonated species in positive ion modes, and when undertaking collision-induced dissociation, a common cleavage was described.[42,52] Fentanyl analogues, with a 4-anilidopiperidine structure, in a collision cell show a specific cleavage C–N between the piperidine ring and the amide group commonly resulting in the peak of the carbocation *m/z* 188.10 which is subsequently fragmented to *m/z* 105.06. The same fragmentation[42] was observed for peaks with *m/z* 84.08 for norfentanyl and acetyl norfentanyl, *m/z* 268.17 for alfentanil, *m/z* 228.1233 for remifentanil, *m/z* 238.1264 for sufentanil, *m/z* 246.17 for carfentanil and *m/z* 156.10 for *N*-methylcarfentanil. The other proposed main fragmentation[42] corresponds to a degradation of the piperidine ring due to cleavages on both the C(2)–N and C(6)–N bonds of the ring.

Strayer *et al.*[53] were able to detect 24 illicitly manufactured fentanyl analogues and metabolites in whole blood at a low concentration ranging between 0.1 – 0.5 ng/mL using a validated assay on a 6420 triple quadrupole LC-MS/MS system (Agilent Technologies). Separation was achieved with a Raptor biphenyl analytical column (150.0 × 3.0 mm, 2.7 µm) with a linear gradient between A: 10.0 mM ammonium formate and 0.1% FA in water and B: 0.1% formic acid in acetonitrile (ACN). The elution program was follows: 10% B (0 – 2 min), 10 – 90% B (2 – 8 min), 90% B (8 – 8.5 min), 90 – 10% B (8.5 – 8.6 min) and held at 10% until 13.5 min. Electrospray ionization in a positive-ion scan mode and a dynamic MRM scan function were applied. Unfortunately, according to the author, separation and identification between isomeric species could not be achieved under these conditions (*e.g.* butyryl fentanyl/isobutyryl fentanyl and *para*-fluorobutyryl fentanyl/4-fluoroisobutyryl fentanyl). Another comprehensive analytical method was proposed by Fogarty *et al.*[54] in which fentanyl and 18 novel fentanyl analogues and metabolites were evaluated in peripheral blood by LC-MS/MS. The instrumentation consisted of a Xevo TQ-S Micro coupled with an Acquity UPLC (both from Waters). Chromatographic separation was achieved for all isobaric compounds using an Agilent Poroshell EC C-18 column (3.0 × 150 mm, 2.7 µm) with a linear gradient of between A: 5 mM ammonium formate (pH 3) and B: 0.1% FA in methanol. The gradient was the following: 40 – 45% B (0 – 7 min), 45 – 90% (7 – 7.1 min), 90% B (7.1 – 8), 90 – 45% B (8 – 8.1) and held to 40% B until 9 min. The instrumentation was operated using the positive-ion electrospray in MRM mode. Such triple quadrupole-based methods are highly sensitive and provide a robust

Table 2 Analytical techniques used for the quali-quantitative determination of fentanyl-like compounds; *screening* means a method comprehensive of multiple fentanyl-like drugs

Detection	Instrument	LOQ[a]	LOD[b]	Fentanyl analogues	Sample	Column	Phases	Ref.
GC-FID[c]	Varian CP-3800 GC (Palo Alto, CA, USA) equipped with an FID detector	—	0.01 – 0.07	Sufentanil and alfentanil	Plasma and urine	CP-Sil8 fused-silica capillary column	—	44
GC-MS[d]	An Agilent 6890 GC (Agilent Technologies, Inc., Wilmington, DE) equipped with a 5973 MSD	—	—	Acetyl fentanyl	Blood	—	—	13
GC-MS	An Agilent Technologies 7890A series gas chromatograph coupled with a 5975C mass spectrometer	125	62.5	Acetyl fentanyl	Post-mortem tissues	RTX-1-ms column	—	40
GC-MS	GC-MS (7890A/ 5975C; Agilent Technologies, Santa Clara, CA, USA)	100	50	Acetyl fentanyl	Blood, urine and vitreous	Zebron ZB-5MS	—	70
GC-MS	GC/MS analysis was performed on a GC/MS-QP2010 (Shimadzu, Kyoto, Japan) equipped	—	—	Acetyl fentanyl	Blood and urine	DB-5 ms column	—	98
GC-MS/MS[e]	Bruker 456-GC gas chromatograph connected to a SCION TQ mass spectrometer (Bruker Daltonics, Billerica, MA, USA)	20 (ng/g)	1 (ng/g)	Acetyl fentanyl	Blood and urine	Rtx-5Sil MS	—	99
GC-MS	6890 gas chromatograph with a 5973 mass spectrometer from Agilent (Santa Clara, CA, USA)	—	—	Acrylfentanyl	Seized capsule	XTI-5 capillary column	—	60
GC-MS	Agilent Technologies (Santa Clara, CA, USA) 7890A/5975C gas chromatograph–mass spectrometer	—	5	Acrylfentanyl	Peripheral blood	DB-1MS column	—	85
GC-MS	Agilent Technologies (Santa Clara, CA, USA) 6890/5973 gas chromatograph–mass spectrometer	0.5	0.04 – 0.08	Alfentanil, sufentanil and fentanyl	Urine	J&W 5% phenylmethyl-syloxane capillary column	—	57
GC-MS	GC-MS (7890A/5975C; Agilent Technologies, Santa Clara, CA, USA) equipped	100	50	Butyr fentanyl	Blood, vitreous and urine	Zebron ZB-5MS	—	70
GC-MS	GC-MS (Agilent Technologies, Santa Clara, CA, USA)	—	—	Furanylfentanyl	Blood, vitreous and urine	HP-5MS	—	100
GC-MS	Hewlett Packard 5890/6890N Series II GC with a 5971A/5973A MS	50	10	Fentanyl	Urine	HP-5MS/DB-1MS	—	47
GC-MS	An Agilent 6890 GC with an Agilent 5973 MSD (Agilent Technologies, Wilmington, DE)	4	1	Fentanyl	Rabbit plasma	HP-5MS		101
GC-MS	—	—	—	Fentanyl	Post-mortem tissues	—	—	102
GC-MS	CP-3800 gas chromatograph connected to a 1200-L mass spectrometer (Bruker, Billerica, MA)	—	—	Methyl-derivatives	Powdered sample	DB-5MS	—	61
GC-MS	An Agilent 7890B gas chromatograph, coupled to a 5977A quadrupole mass spectrometer detector (Agilent, Santa Clara, CA, USA)	—	—	Ocfentanil	Heroin samples	HP-5MS	—	103
GC-MS	GC-MS (AUTOMASS GC-MS system, JEOL, Tokyo, Japan)	—	—	Remifentanil	Blood	InerCap17MS capillary column	—	104
GC-MS	—	—	—	Screening	Blood, vitreous and urine	Rtx-5 capillary column	—	41
GC-MS	An Agilent 7890 gas chromatograph coupled with an Agilent 5975A quadrupole mass selective detector (Agilent Technologies, Milano, Italy)	5 – 200	2 – 100	Screening	Urine	Short GC column 5% phenyl methyl silicone	—	105
GD-FID	Varian CP-3800 system (Palo Alto, CA, USA) equipped with an FID detector	2 – 15	0.6 – 4.5	Alfentanil, sufentanil	Urine	Chrompack CP-Sil 8CB	—	46
Immunoassays	ELISA kits purchased from Immunalysis (Pomona, CA)	—	—	Acrylfentanyl	Blood	—	—	85
Immunoassays	ELISA kits purchased from Immunalysis (Pomona, CA)	—	0.1	Fentanyl	Blood and fresh/ decomposed skeletal tissues	—	—	36
Immunoassays	Homogeneous enzyme immunoassay was performed on the Olympus AU400e automated chemical analyzer	2	1	Fentanyl	Urine	—	—	58
Immunoassays	Fentanyl ready-to-use (RTU) ELISA kits (Product #s 131519 and 131515) were obtained from Neogen® (Lexington, KY)	—	0.25 – 0.5	Fentanyl	Blood and urine	—	—	106
Immunoassays	HEIA (Immunalysis Corporation, Pomona, CA) was performed on the Olympus AU480 analyzer (Beckman Coulter Inc., Brea, CA)	2	—	Fentanyl	Urine	—	—	107
Immunoassays	Thermo DRI fentanyl enzyme immunoassay, the ARK™ fentanyl assay homogeneous enzyme immunoassay, and the Immunalysis Fentanyl Urine SEFRIA Drug Screening Kit	0.5 – 1	—	Screening	Urine	—	—	59
Immunoassays	In-house developed opioid activity reporter assay	—	—	Screening	Blood, urine and vitreous	—	—	108

LC-UV[f]	Hewlett-Packard 1090-II liquid chromatograph (now Agilent, Palo Alto, CA, USA) equipped with a UV-Vis diode array detector	1.1 – 5.5	0.4 – 1.9	Alfentanil, fentanyl, sufentanil	Plasma and urine	C6 reversed-phase	(A) Sodium butane-1-sulfonate in sulfuric acid 30%	45
LC-HR-MS[g]	—	—	1 – 2	Screening	Oral fluid and urine	Zorbax Eclipse C18	(A) Water + FA[h] (B) MeOH[i] + FA	75
LC-HR-MS	An Agilent Technologies 1200 series instrument coupled to a TripleTOF 5600 system (AB Sciex, Concord, Ontario, Canada)	—	—	AH-7921 and MT-45	Various body fluids and tissues	Zorbax Eclipse XDB-C8 column	(A) AmFo[j] + FA (B) MeOH + FA	109
LC-HR-MS-targeted method	An Agilent (Waldbronn, Germany) 1100 HPLC instrument coupled to a Bruker Daltonics (Bremen, Germany) micrOTOF	—	—	Screening	Meconium	Phenomenex Luna PFP (2)	(A) AmAc[k] (B) ACN[l] + FA	92
LC-HR-MS-DIA[m]	A Waters Acquity UPLC (Waters, Milford, MA) coupled to a Sciex 5600 TripleToF (Sciex, Framingham, MA) mass spectrometer set	—	—	U-47700 and metabolites	Urine	Poroshell 120 EC-C18	(A) AmAc + FA (B) MeOH + FA	32
LC-HR-MS-AIF[n]	TF Q-Exactive Plus system equipped with a heated electrospray ionization (HESI)-II source coupled to a ThermoFisher Scientific (TF, Dreieich, Germany) Dionex UltiMate 3000 HPLC	0.25	0.1	Screening	Plasma	Accucore phenyl-hexyl	(A) AmFo + FA (B) MeOH + ACN + FA	17
LC-HR-MS-DDA[o]	Thermo Fischer Ultimate 3000 UHPLC system coupled to a Sciex 6600 QTOF system	—	—	Butyr fentanyl	Blood and urine	Synergy Polar RP column	(A) AmFo + FA (B) ACN + FA	87
LC-HR-MS-DDA	Thermo XRS UHPLC system, interfaced to a Thermo Q Exactive Focus mass spectrometer, operating in heated positive ion electrospray mode	—	—	Screening	Blood, urine and vitreous	Atlantis T3 HPLC	(A) AcA + water (B) AcA + ACN	1
LC-HR-MS-DIA	Acquity UPLC system (Waters Corporation, Milford, USA) coupled to SYNAPT G2 (Waters MS Technologies, Manchester, UK) TOF mass spectrometer	—	0.001 – 0.005 (mg/kg)	Screening	Blood	Acquity UPLC BEH C18	(A) Water + FA (B) ACN	55
LC-HR-MS-DIA	ACQUITY UHPLC system from Waters Corporation (Milford, MA, USA) with Xevo G2-S QTOF (Waters MS Technologies, Manchester, UK)	0.001 – 0.005 (mg/kg)	0.0005 – 0.001 (mg/kg)	Screening	Blood	ACQUITY UHPLC HSS C18 column	(A) AmFo (B) ACN + FA	42
LC-HR-MS-DIA	Agilent 1290 Infinity UHPLC system with an Agilent 6550 iFunnel QTOF mass spectrometer	—	—	Screening	Urine	Acquity HSS T3	(A) AmFo + FA (B) ACN + FA	110
LC-MS-LIT[p]	A Thermo Scientific Dionex Ultimate 3000 RSLC ultra high-performance liquid chromatograph (Idstein, Germany) coupled to a Bruker Daltonics AmaZon Speed ion trap mass spectrometer (Bremen, Germany)	—	0.1 – 0.5	Screening	Blood and urine samplesand/or tissue homogenates	Acclaim RSLC 120 C18 column	(A) AmFo + FA (B) AmFo + FA + ACN	89
LC-MS-LIT	A LTQ XLTM linear ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA)	—	—	Acetyl fentanyl	Cardiac blood, gastric contents and urine detected	Hypersil Gold	(A) AmAc (B) MeOH	98
LC-MS-LIT	—	—	—	Fentanyl	Goat blood	HALO C18	(A) Water + FA (B) ACN + FA	111
LC-MS-LIT	An Accela LC system (Thermo Fisher Scientific, TF, Dreieich, Germany) coupled to the TF LXQ LIT	—	10	3-Methylfentanyl and isofentanyl	Rat urine	TF Hypersil GOLD	(A) Water + FA (B) ACN + FA	112
LC-MS/MS[q]	HPLC 1260 Infinity system coupled to a 6420 triple quadrupole	0.1 – 0.25	0.02 – 0.1	Screening	Blood	Raptor biphenyl	(A) AmFo + FA (B) ACN + FA	53
LC-MS/MS	An Agilent 1260 liquid chromatograph system couplet to 6460 triple quadrupole mass spectrometer with a Jetstream electrospray source (Agilent Technologies, California, USA)	—	0.01 – 0.1	Screening	Blood and urine	Poroshell EC-C18	(A) AmFo + FA (B) ACN + FA	30
LC-MS/MS	An ABSciex QTrap 4000 tandem mass spectrometer (Darmstadt, Germany) coupled to a LC-20 (Shimadzu, Jena, Germany)	1	—	Fentanyl and norfentanyl	Urine	Zorbax Eclipse C18	(A) AmFo + FA (B) ACN + FA	76
LC-MS/MS	Shimadzu Nexera X2 30 AD UPLC (Shimadzu, Melbourne, Australia) coupled to a Sciex 4500 Q-Trap (Sciex, Melbourne, Australia)	—	0.001 (ng/mg)	Screening	Hair	Kinetex C18	(A) AmFo + FA (B) ACN + FA	35
LC-MS/MS	Agilent 1290 Infinity system UPLC coupled to an Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara,CA, USA)	0.2 – 2	0.05 – 0.5	Screening	Dried blood sample	Kinetex C18	(A) AmFo (B) MeOH/ACN + FA	33
LC-MS/MS	Agilent LC 1100 coupled to an AB/MDS Sciex 3200 QTrap LC-MS/MS	0.1 – 0.3	0.05 – 0.2	Screening	Blood and urine	Gemini C18	(A) AmAc (B) ACN + FA	39
LC-MS/MS	Shimadzu Prominence HPLC system (Shimadzu USA Manufactoring Inc., Canby, OR, USA) couplet to a Sciex 3200 Q TRAP LC-MS/MS System (Applied Biosystems, Concord, ON, Canada)	—	—	Screening	Meconium	Genesis C18	(A) AmAc (B) ACN + FA	92

LC-MS/MS	A Waters (Milford, MA, USA) Acquity UPLC I-Class coupled to a Xevo® TQ-S micro LC-MS-MS	—	—	Screening	Blood and urine	Poroshell 120 EC-C18	(A) AmFo (B) MeOH + FA	113
LC-MS/MS	A Waters Xevo TQD LC/MS mass spectrometer attached to a ACQUITY UPLC® System controlled by MassLynx software (Milford, MA)	1	—	Screening	Blood, urine and vitreous	Allure Biphenyl	(A) AmFo + FA (B) MeOH	91
LC-MS/MS	A Nexera UHPLC system coupled to a LCMS-8050 mass spectrometer from Shimadzu (Marlborough, MA, USA)	0.1	—	Screening	Dried blood spot	Raptor Biphenyl	(A) AmFo + FA (B) MeOH	68
LC-MS/MS	An Agilent LC coupled to an API 4000 tandem quadrupole mass spectrometer as detector (Applied Biosystems, Foster City, CA)	0.1 - 0.25	—	Screening	Plasma, blood and dried blood spot	Eclipse XDB-C8	(A) Water + FA (B) MeOH	82
LC-MS/MS	UPLC-MS/MS system from Waters Chromatography B.V. (Etten-Leur, The Netherlands) consisted of a Waters Aquity UPLC Sample Manager coupled to a Waters TQ Detector	0.1 - 0.2	—	Fentanyl and norfentanyl	Plasma	Acquity BEH C18	(A) Water + AmFo + FA (B) MeOH + FA	37
LC-MS/MS	UHPLC was performed using an UPLC separation module (Waters, Milford, MA, USA) coupled to a Quattro Premier tandem mass spectrometer (Waters)	3	—	Fentanyl and norfentanyl	Urine	BEH Phenyl	(A) Water + FA (B) MeOH + FA	96
LC-MS/MS	An Agilent 1100 series HPLC coupled to an Agilent 6430 triple quadrupole tandem mass spectrometer (Santa Clara, CA)	1	—	U-47700, U-50488 and furanyl fentanyl	Blood, urine and vitreous	Zorbax Eclipse C18	(A) Water + FA (B) MeOH + FA	90
LC-MS/MS	Alliances HT 2795 LC system coupled to a Quattro Premier mass spectrometer (Waters, Milford, USA)	0.1 - 0.2	—	Screening	Plasma and urine	XTerra MS C18	(A) Water + FA (B) ACN + FA	73
LC-MS/MS	LC Symbiosis system coupled to mass spectrometry detection was carried out in ESI mode using an API 5500 (AB-Sciex, Concord, Ontario, Canada) triple quadrupole	0.5	—	Fentanyl and metabolites	Plasma	XTerra MS C18	(A) Water + FA (B) ACN + FA	77
LC-MS/MS	LC Symbiosis system coupled to Analytes were detected using an Applied Biosystems API 5500 Triple Quadrupole MS (Foster City, CA)	—	0.002 - 0.04	Screening	Urine	XTerra MS C18	(A) Water + FA (B) ACN + FA	78
LC-MS/MS	LC-MS/MS analyses were performed using an Agilent Technologies UPLC 1290 coupled with a 6490 Triple Quad from Agilent Technologies (Santa Clara, CA, USA)	0.1 - 2	0.02 - 0.6	Screening	Serum, plasma and post-mortem tissues	Zorbax Eclipse phenyl-hexyl	(A) Water + AmFo (B) ACN + FA	26
LC-MS/MS	API 4000 tandem mass spectrometer (AB Sciex, Darmstadt, Germany) interfaced to a binary LC pump (series 1100, Agilent, Waldbronn, Germany)	0.02 - 0.7	0.01 - 0.2	Screening	Plasma and urine	Luna C18	(A) MeOH/ ACN/AmAc	74
LC-MS/MS	A Waters Xevo TQ-S Micro coupled with a Waters Acquity UPLC (Milford, MA)	0.1	—	Screening	Blood	Poroshell EC C-18	(A) AmFo (B) MeOH + FA	54
LC-MS/MS	A Thermo Scientific Dionex UltiMate 3000 Rapid Separation LC system (Idstein, Germany) coupled to a Thermo Scientific TSQ Vantage triple quadrupole tandem mass spectrometer (Thermo Fisher Scientific, San Jose, CA)	0.1 - 1	—	Screening	Blood and vitreous	Kinetex F5	(A) Water + FA (B) ACN + FA	31
LC-MS/MS	Waters Acquity UPLC system (Milford, MA, USA) coupled to a Waters TQD tandem mass spectrometer (Waters Corp., Milford, MA, USA)	0.05 - 0.5	0.01 - 0.25	Screening	Blood, serum, plasma and urine	Zorbax RX-SIL	(A) AmFo (B) ACN	27
LC-MS/MS	Accela UHPLC system coupled to a TSQ Quantum Access (Thermo- Fisher (TF) Scientific, Dreieich, Germany) mass spectrometer	—	—	Screening	Plasma	Hypersil Gold Phenyl	(A) AmFo + FA (B) ACN + FA	38
LC-MS/MS	Accela UHPLC coupled to a TSQ Quantum Access MS (Thermo-Fisher Scientific, Dreieich, Germany)	2 - 15	—	Screening	Plasma	Hypersil Gold C18	(A) ACN + FA	43
LC-MS/MS	An Acquity UPLC coupled to a Quattro Premiere Xe tandem mass spectrometer from Waters (Milford, MA)	0.14	0.044	Fentanyl	Blood	Acquity BEH C18	(A) AmFo (B) MeOH	114
LC-MS/MS	Waters Acquity Ultra-Performance Liquid Chromatograph (UPLC) coupled to a Waters TQ-D detector	—	—	Acetyl fentanyl	Blood, liver, vitreous and urine	UPLC BEH T3	(A) Water + FA (B) ACN + FA	115
LC-MS/MS	LC-MS was performed with a Shimadzu LC-MS8040 (Shimadzu Corporation, Kyoto, Japan)	—	—	Acetyl fentanyl	Blood, urine and gastric contents	Shim-pack FC-ODS	(A) AmFo (B) MeOH	116
LC-MS/MS	A Waters Acquity UltraPerformance Liquid Chromatograph coupled to a Waters Quattro Premier XE tandem mass spectrometer	0.1	0.05	Acrylfentanyl	Blood	Acquity UPLC BEH C18	(A) Water + FA (B) ACN + FA	85

LC-MS/MS	LC-20AC pumps (Shimadzu, Columbia, MD) interfaced with an API 3200 triple-quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA)	0.25	—	Alfentanil	Plasma	Sunfire C18	(A) Water + AcA (B) ACN + AcA	97
LC-MS/MS	A Waters Xevo TQD LC/MS mass spectrometer attached to an ACQUITY UPLC System controlled by MassLynx software (Milford, MA)	1	—	Butyr fentanyl, acetyl fentanyl and acetyl norfentanyl	Blood, vitreous humor, gastric contents, brain, liver, bile and urine.	Allure Biphenyl 5 µm	(A) AmFo + FA (B) MeOH	86
LC-MS/MS	A Sciex 3200 QTRAP mass spectrometer coupled to an Agilent 1260 LC system (Sciex, Cheshire, UK)	0.05	—	Carfentanil	Blood	C18 column	(A) Water + FA (B) ACN	69
LC-MS/MS	Waters Acquity Ultra Performance Liquid Chromatograph coupled to a Waters Quattro Premier XE tandem mass spectrometer	0.01	—	Carfentanil	Blood	Waters Acquity UPLC BEH C18	(A) Water + FA (B) ACN	79
LC-MS/MS	An Agilent6460 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA)	0.5	—	Carfentanil	Rat plasma	Poroshell 120 SB-C18	(A) Water + FA (B) ACN	80
LC-MS/MS	The HPLC system (Shimadzu, Japan) consisted of LC-20 AD coupled to an API 3000 (Applied Biosystems, USA)	0.02	—	Fentanyl	Plasma	Capcell Pak C18 MG II	(A)ACN + water + FA	117
LC-MS/MS	The HPLC system (Shimadzu, Japan) consisted of LC-20 AD coupled to an API 3200 (Applied Biosystems, USA)	0.03 – 0.04	—	Fentanyl and norfentanyl	Plasma and saliva	Alltima C18	(A) Water + FA (B) ACN + MeOH + FA	34
LC-MS/MS	The LC system (Shimadzu, Kyoto, Japan) consisted of an LC-10AT pump coupled to Finnigan Model TSQ-7000 triple-quadrupole (Thermo Fisher Scientific, Waltham, MA, USA)	0.05	—	Fentanyl	Plasma	TSKgel ODS-100V	(A) Water + FA (B) ACN + FA	88
LC-MS/MS	Alliance HPLC 2695 separation module (Waters, Milford, MA, USA) coupled to a tandem mass spectrometer Quattro micro (Waters, Milford, MA, USA)	0.2 – 0.25	—	Fentanyl	Newborn pig plasma and cerebrospinal fluid samples	Luna C18	(A) Water + FA (B) ACN + FA	118
LC-MS/MS	LC Prominence (Shimadzu, Kyoto, Japan) coupled to API 4000 tandem mass spectrometry system (AB Sciex, Framingham, MA, USA)	0.05	—	Fentanyl	Plasma	Inertsil ODS-3	(A) Water + FA (B) ACN + FA	119
LC-MS/MS	LC-MS/MS analysis was carried out using a Shimadzu system LC-20ADXR (Shimadzu Prominence, Antwerpen, Belgium) in combination with a 3200 QTRAP (Applied Biosystems, Halle, Belgium)	0.0025 – 0.005	0.3 – 5 (pg/mL)	Fentanyl and norfentanyl	Urine and whole blood	Acquity C18	(A) Water + FA (B) ACN + FA	120
LC-MS/MS	A LC-MSMS (Waters, Acquity UPLC, Xevo TQ-S)	—	—	Fluorofentanyl isomers	Serum	Chiral column CHIROBIOTIC	(A) MeOH + FA + NH_3	83
LC-MS/MS	LC-30AD liquid chromatography system, (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a Triple Quad 4500 System (AB SCIEX Instruments, Concord, Ontario)	0.01 (ng/g)	—	Acrylfentanyl	Blood	Acquity UPLC BEH Phenyl	(A) AmFo + FA (B) MeOH + FA	71
LC-MS/MS	An Agilent 1100 series, HPLC chromatograph (Agilent Technologies, Palo Alto, USA) and an Esquire 3000 plus mass spectrometer MRM (Bruker Daltonic GmbH, Bremen, Germany)	—	—	Ocfentanil	Heroin sample	Poroshell 120 EC-C18	(A) AmFo + FA (B) ACN + FA	103
LC-MS/MS	The UPLC-MS/MS analysis was performed using an Acquity separations module coupled to the Acquity TQD mass detector equipped with ES interface (Waters Milford, MA, USA)	2	—	Ocfentanil	Kidney, liver, stomach content (semi-solid), bile and brain tissue and swab of the mucous membrane of the nose	Acquity UPLC HSS C18 column	(A) Water + FA (B) ACN + FA	72
LC-MS/MS	LC Acquity system (Waters, Manchester, UK) coupled to an API 4000 mass spectrometer (ABSciex, Toronto, Ontario, Canada)	0.05	0.01	Ocfentanil	Blood, gastric content, bile, vitreous and nose swabs	ACQUITY HSS C18 column	(A) Water + FA (B) ACN + FA	121
LC-MS/MS	An Accela LC (Thermo Scientific, Waltham, MA) coupled to a triple quadrupole mass spectrometer (TSQ Quantum)	0.05	—	Remifentanil	Plasma	Kinetex C18	(A) Water + FA (B) MeOH	48
LC-MS/MS	All experiments were performed on an Agilent 6460A (Santa Clara, CA) triple quadrupole LC-MS/MS system, with a combined Agilent 1200 series LC system	0.2	—	Remifentanil	Whole blood and plasma	3-µm HyPURITY C18	(A) Water + FA (B) MeOH	81
LC-MS/MS	A Waters 2695 separation module (Waters Co., Milford, MA, USA) and a Quattro micro triple-quadrupole mass spectrometer (Waters)	0.17	0.10	Remifentanil	Rat plasma	Chromolith Performance RP-18 monolithic column	(A) AmAc + ACN	84
LC-MS/MS	A Waters Alliance HPLC system (Waters, Eschborn, Germany) coupled to a Waters Quattro Micro tandem mass spectrometer	0.005	—	Sufentanil	Plasma	Kinetex C18	(A) Water + ACN + TFA	93

LC-MS/MS	A Waters Alliance HPLC system (Waters, Eschborn, Germany) coupled to a Waters Quattro Micro triple quadrupole mass spectrometer	—	—	Sufentanil	Blood	Kinetex C18	(A) Water + ACN + TFA	94
LC-MS/MS	A Waters ACQUITY UPLC system coupled to a Micromass Quattro Premier XE ES mass spectrometer (Waters Corp., Milford, MA, USA)	0.07	—	Sufentanil	Plasma	ACQUITY UPLC BEH C18 column	(A) ACN + water	95
LC-MS/MS	1260 Infinity LC coupled to a 6460 tandem mass spectrometer (Agilent Technologies, Santa Clara, CA)	1	—	U-47700	Urine	Poroshell 120 EC-C18	(A) AmAc + FA (B) MeOH + FA	32

a. LOQ, Limit of quantification expressed in ng/mL; in the case of screening method LOQ values are indicated as range among the substances studied. b. LOD, Limit of detection expressed in ng/mL; in the case of screening method LOD values are indicated as range among the substances studied. c. GC-FID, Gas-chromatography coupled to flame ionization detector. d. GC-MS, Gas-chromatography coupled to single quadrupole mass spectrometer. e. GC-MS/MS, Gas-chromatography coupled to triple quadrupole mass spectrometer. f. LC-UV, Liquid-chromatography coupled to ultraviolet detector. g. LC-HR-MS, Liquid-chromatography coupled to a high-resolution mass spectrometer. h. FA, Formic acid. i. MeOH, Methanol. j. AmFO, Ammonium formate. k. AmAc, Ammonium acetate. l. ACN, Acetonitrile. m. LC-HR-MS-DIA, Liquid-chromatography coupled to a high-resolution mass spectrometer operating in data independent acquisition mode. n. LC-HR-MS-AIF, Liquid-chromatography coupled to a high-resolution mass spectrometer operating in all-ion fragmentation mode. o. LC-HR-MS-AIF, Liquid-chromatography coupled to a high-resolution mass spectrometer operating in data dependent acquisition mode. p. LC-MS-LIT, Liquid-chromatography coupled to linear ion trap mass spectrometer. q. LC-MS/MS, Liquid chromatography coupled to triple quadrupole mass spectrometer.

quantification, although they have some drawbacks. The addition of new compounds usually needs a thorough mass spectrometry (MS) optimization, and the total number of monitored compounds is limited. Moreover, they also do not allow for the retrospective evaluation of MS data to identify formerly unknown or unexpected compounds. The alternative is represented by high-resolution mass spectrometry (HR-MS), which provides a contemporaneous nontargeted acquisition of precursor-ions and product-ions at both high resolution and high mass accuracy. LC-HR-MS has emerged as a fundamental method for the detection of novel synthetic opioids, nevertheless, this technology is not sustainable in most forensic laboratories. Hikin *et al.*[1] developed a method for the detection and semi-quantitation of synthetic fentanyl analogues using a data-dependent approach in HR-MS. The instrumentation was comprised of a Thermo XRS Ultra High-Performance Liquid Chromatography system interfaced to a Thermo Q Exactive Focus operating in a heated positive-ion electrospray mode. Chromatographic separation was achieved on an Atlantis T3 column (Waters) using a gradient consisting of A: 0.1% acetic acid and B: ACN containing 0.1% acetic acid. The full-scan mode operated at a mass resolution of 70000 across a mass range of 50 – 750 amu, whereas data-dependent scanning was enabled utilising an inclusion list of over 800 compounds. By contrast, Pedersen *et al.*[55] presented a UHPLC-TOF-MS method with data-independent acquisition over a *m/z* 50 – 1200 range capable to distinguish either common drugs of abuse as well as new designer drugs and hardly occurring ones.

GC-MS was the gold standard for the previous decade. It was appreciated for untargeted data acquisition coupled with library searching for compounds detected in biological specimens. Diagnostic ions of fentanyl analogues in GC/EI-MS are: 1) M^+ as base peaks for *N*-benzylated and *N*-methylated analogues, 2) M-91 coming from an elimination of the benzyl fragment, and 3) ions originated from cleavages of the piperidine ring and elimination of the propionyl group, such as *m/z* 146, 160, 164, 180 and 177.[56] Strano-Rossi *et al.*[57] developed a rapid and sensitive method for the simultaneous determination of alfentanil, sufentanil and fentanyl in urine. GC/EI-MS analyses were performed in an Agilent 6890 Gas Chromatograph coupled with an Agilent 5973 mass-selective quadrupole detector. The GC was equipped with a J&W 5% phenylmethylsyloxane capillary colum of 30 m × 0.25 mm. i.d., 0.50 µm film thickness. The analysis was performed using both a full-scan (*m/z* 50 – 500) and a selected ion-monitoring mode. A derivatization step with pentafluoropropionic anhydride (PFPA) was proposed prior analysis in order to magnify the intensity of nor-metabolites. Even though, in many cases, fentanyls can be analyzed in GC without a derivatization step, since they show inherently appreciable mass spectra. In addition, the use of derivatizing agents is destructive for a stationary phase packaged in the chromatographic column, therefore seems to be suitable to limit their use only when firmly required. However, due to a lack of sensitivity and extensive sample preparation, GC-MS was displaced by targeted LC-MS/MS methods.[15] Occasionally, LC-UV and GC-FID are still used.

Immunoassays are indeed becoming more successful. They are in continuous development while trying to obtain ever more analytical sensibility, as well as large selectivity for the highest number of compounds. The strength of this method relies on the ease of use, so that not highly qualified staff members are required, and on its fast response. In general, immunoassays can be used by law enforcement to check whether people are under the influence of drugs, for example, while they are driving vehicles. In any case after immunoassay screening, more in-depth investigations are obviously required. None of these drugs is revealed in the standard urine opiate immunoassays. However, specific commercial immunoassays for fentanyl[58] and some designer fentanyl in urine are available: Thermo DRI Fentanyl Enzyme Immunoassay, the ARK Fentanyl Assay homogeneous enzyme immunoassay and the Immunalysis Fentanyl Urine SEFRIA Drug Screening Kit. These demonstrated a good detectability of designer fentanyls in urine samples from authentic acute intoxications compared to LC-HR-MS, as a reference method.[59]

NMR and IR spectroscopy are reported only for detection in powder from seized samples.[60,61] In NMR most of the designer opioids share signature signals attributed to piperidine protons and aromatic protons in the regions of 2.8 – 3.7, 4.5 – 5.0, and 7.0 – 8.0 ppm.[62,63] Raman spectroscopy can be successfully applied in forensic science, to reveal the chemical identity of fentanyl-like compounds, in a sensitive and straightforward approach.[64-67] In Table 3 analytical characteristics of fentanyl-like compounds are discussed and compared.

4 Conclusions

The present work dealt with examining fentanyl-related publications of the last decade, between 2008 and 2018, reaching the maximum in the last two years (Fig. 3). From this research, it emerged that the most common methods to extract and detect

Table 3 Summary of analytical characteristics of fentanyl-like compounds

Analyte	Chemical formula	Monoisotopic mass	GC/MS base peaks	MRM transition (ESI +)	References
2-Fluorobutyryl fentanyl	$C_{23}H_{29}FN_2O$	368.22639	277, 164, 207	369.2 > 299.1	42, 122
2-Fluorofentanyl	$C_{22}H_{27}FN_2O$	354.21074	263, 164, 207	355.3 > 105.1	27, 122
2-Thiophenoyl fentanyl	$C_{24}H_{26}N_2OS$	390.17658	111, 299, 256	—	122
3-Methylfentanyl	$C_{23}H_{30}N_2O$	350.23581	259, 160, 203	351.5 > 202.4	73, 122
4-ANPP	$C_{19}H_{24}N_2$	280.19394	146, 189	281.2 > 105.0	27, 122
4-Fluorobutyryl fentanyl	$C_{23}H_{29}FN_2O$	368.22639	277, 164, 207	369.3 > 188.1	27, 122
4-Fluoroisobutyryl fentanyl	$C_{23}H_{29}FN_2O$	368.22639	277, 164, 207	—	122
4-Methoxyacetylfentanyl	$C_{22}H_{28}N_2O_2$	352.21508	—	353.2 > 281.2	42
4-Methoxybutyrfentanyl	$C_{24}H_{32}N_2O_2$	380.24637	289, 176, 219, 290	381.2 > 311.2	42, 122
4-Methoxymethylfentanyl	$C_{24}H_{32}N_2O_2$	380.24637	—	381.2 > 325.2	42
Acetyl fentanyl	$C_{21}H_{26}N_2O$	322.20451	146, 231, 188	323.4 > 188.2	31, 122
Acetyl norfentanyl	$C_{13}H_{18}N_2O$	218.14191	82, 83, 175	219.1 > 177.1	42, 122
Acrylfentanyl	$C_{22}H_{26}N_2O$	334.20451	—	335.2 > 105.0	27
AH-7921	$C_{16}H_{22}Cl_2N_2O$	328.11091	126, 127, 173	329.1 > 284.0	122
Alfentanil	$C_{21}H_{32}N_6O_3$	416.25359	—	417.3 > 268.6	73
Alpha-methylacetylfentanyl	$C_{22}H_{28}N_2O$	336.22016	245, 246, 91, 110	337.2 > 295.2	122
Benzoylbenzyl fentanyl	$C_{25}H_{26}N_2O$	370.20451	91, 105, 265	—	122
Benzylfentanyl	$C_{21}H_{26}N_2O$	322.20451	—	323.2 > 267.2	42
Beta-hydroxy-thiofentanyl	$C_{20}H_{26}N_2O_2S$	358.17150	245, 146	359.2 > 192.1	27, 122
Beta-hydroxyfentanyl	$C_{22}H_{28}N_2O_2$	352.21507	—	353.2 > 279.2	42
Butyr-fentanyl	$C_{23}H_{30}N_2O$	350.23581	146, 259, 189	351.1 > 105.0	27, 122
Carfentanyl	$C_{24}H_{30}N_2O_3$	394.22564	303, 304, 187, 105	395.4 > 335.3	31, 122
Cyclopentyl fentanyl	$C_{25}H_{32}N_2O$	376.25146	285, 189, 146	377.2 > 281.2	42, 122
Cyclopropyl fentanyl	$C_{23}H_{28}N_2O$	348.22016	257, 146, 189, 69	349 > 188	122, 123
Fentanyl	$C_{22}H_{28}N_2O$	336.22016	245, 146, 189, 202	337.2 > 188.5	73, 122
Furanyl fentanyl	$C_{24}H_{26}N_2O_2$	374.19942	95, 283, 240	375.2 > 188.2	31, 122
Hexanoyl fentanyl	$C_{25}H_{34}N_2O$	378.26711	287, 146, 189	—	122
Isobutyryl fentanyl	$C_{23}H_{30}N_2O$	350.23581	259, 146, 189	351.2 > 282.2	42, 122
Lofentanyl	$C_{25}H_{32}N_2O_3$	408.24129	—	409.2 > 353.2	42
Methylcarfentanil	$C_{17}H_{24}N_2O_3$	304.17869	—	305.1 > 249.2	42
Mirfentanyl	$C_{22}H_{24}N_4O_2$	376.18992	—	377.2 > 283.2	42
MT-45	$C_{24}H_{32}N_2$	348.25654	257, 91	349.1 > 181.0	27, 122
Norfentanyl	$C_{14}H_{20}N_2O$	232.15756	—	233.2 > 84.1	73
Ocfentanil	$C_{22}H_{27}FN_2O_2$	370.20565	279, 45, 105, 176	371.2 > 299.2	42, 122
Remifentanil	$C_{20}H_{28}N_2O_5$	376.19982	—	377.1 > 317.0	73
Sufentanil	$C_{22}H_{30}N_2O_2S$	386.20280	—	387.6 > 238.2	73
Thenylfentanyl	$C_{19}H_{24}N_2OS$	328.16093	—	329.1 > 273.1	73
THF fentanyl	$C_{24}H_{30}N_2O_2$	378.23072	287, 146, 71, 189	379.2 > 281.2	42, 122
Thiofentanyl	$C_{20}H_{26}N_2OS$	342.17658	—	343.1 > 287.1	42
Trefentanyl	$C_{25}H_{31}FN_6O_2$	466.24925	—	467.2 > 411.2	42
U-47700	$C_{16}H_{22}Cl_2N_2O$	328.11091	84, 125, 58, 71	329.2 > 284.1	27, 122
U-49900	$C_{18}H_{26}Cl_2N_2O$	356.14221	112, 153	357.2 > 284.0	122, 124
U-50488	$C_{19}H_{26}Cl_2N_2O$	368.14222	—	369.2 > 298.1	27
Valeryl fentanyl	$C_{24}H_{32}N_2O$	364.25146	146, 273, 189	365.2 > 188.2	27, 122

fentanyl-like compounds are SPE or LLE and LC-MS/MS (Fig. 4). In the past few years, gas chromatography was the most common method, but it was irredeemably replaced by LC for owing to its greater flexibility, accuracy and efficiency. In addition to the classic chromatographic techniques, immunoassays methods have good potential for development, since they can be used by non-qualified staff as first-step screening tests in the toxicological surveys.

The most common analyzed matrices are blood or plasma and urine. Generally, the internal standards used are the commercially available deuterated analogues. In our research, we realized that many studies published in the analytical field are American (39/100). Concerning the European situation (42/100): the Scandinavian region, Belgium and Germany are the most active countries in this research area (Fig. 5).

To conclude, we hope that in the coming years in Italy and throughout the rest of Europe more attention will be placed on the fentanyl-issue. This fact, unfortunately, affects us as well as the rest of the world, since the fentanyl emergency is a problem that must be tackled on a global scale.

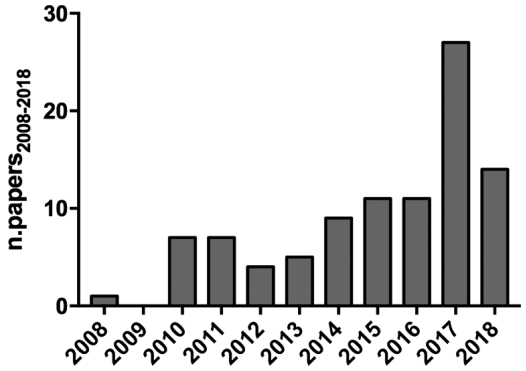

Fig. 3 Trend of papers published in the last ten years in the field of fentanyl-like compounds.

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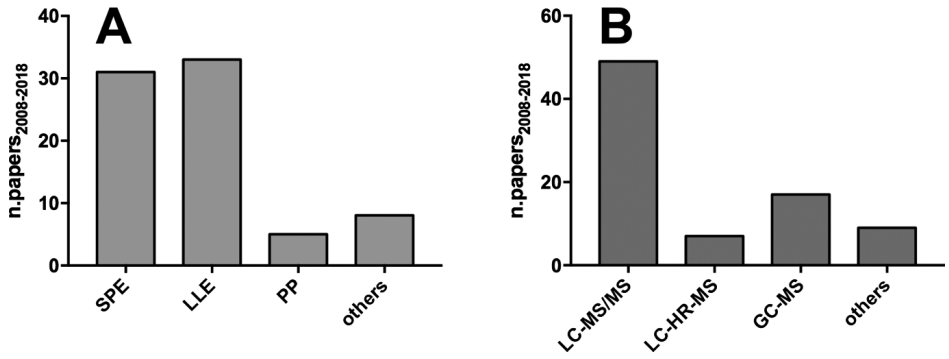

Fig. 4 Number of papers published in the last ten years in the field of fentanyl-like compounds: comparison of extraction techniques (A) and analytical procedures (B).

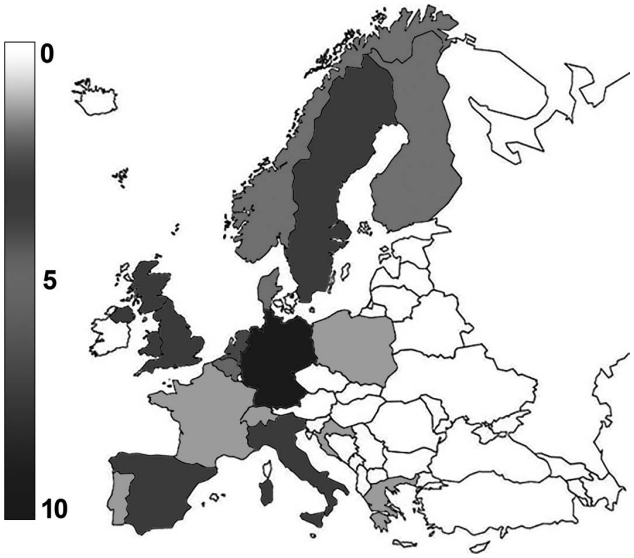

Fig. 5 European distribution of papers published in the last ten years in the field of fentanyl-like compound.

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