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Purity estimation of seized stimulant-type new psychoactive substances without reference standards by nitrogen chemiluminescence detection combined with GC-APCI-QTOFMS

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Highlights

- Reference standards for new psychoactive substances (NPS) are not readily available
- Nitrogen chemiluminescence detection (NCD) possesses equimolar response to nitrogen
- GC-NCD allowed purity estimation of stimulant NPS with only two external calibrators
- The grand mean equimolarity of twenty-eight stimulants studied was 91.9%
- This platform is potentially applicable to instant purity assessment of seized NPS

Abstract

Purity assessment of seized material containing new psychoactive substances (NPS) is complicated without appropriate primary reference standards. Here we present a method for fast quantitative estimation of stimulant-type NPS with use of secondary reference standards, based on gas chromatography nitrogen chemiluminescence detection coupled with atmospheric pressure chemical ionization quadrupole time-of-flight mass spectrometry (GC-NCD-APCI-QTOFMS). Quantification was based on the detector's *N*-equimolar response to nitrogen and using two external nitrogen-containing calibrators, MDMA for *prim*- and *sec*- amines and *α*-PVP for *tert*- amines. Sample preparation involved dissolving the seized powdery material in an organic solvent mixture followed by acylation with *N*-methyl-bis-trifluoroacetamide (MBTFA). The method's between-day accuracy and precision over a five-day period was measured for twenty-eight stimulants: the grand mean equimolarity was 91.9% (CV 5.5%), as compared with primary reference standards. The GC-NCD-APCI-QTOFMS method was applied to the purity estimation of forty-two seized powder samples previously found to contain stimulant-type NPS by appropriate methods. The quantitative results were compared to those obtained by an established method relying on liquid chromatography chemiluminescence detection (LC-CLND), the latter using caffeine as an external calibrator. The mean difference of purity values between the methods was 8.1% (range 0.4 - 26.7%). The presented method might find use as a tool for instant purity assessment in forensic laboratories.

Keywords

Seized drugs
New psychoactive substances
Illicit stimulants
Reference standard
Nitrogen chemiluminescence detection
Time-of-flight mass spectrometry

1. Introduction

Many new psychoactive substances (NPS) are marketed as legal alternatives to replace prohibited drugs of abuse but limited and misleading information regarding to content, purity and pharmacological properties of the sold NPS have been associated with harmful effects for health which may in some cases lead to fatalities [1,2]. The last two decades have shown continuous emergence of NPS on the European illicit drug market: between years 1997 and 2018, more than 730 NPS were reported through the EU Early Warning System [2]. Reference standards are normally required for the identification and quantification of toxicologically relevant

substances. However, the increasingly high number of different NPS is driving forensic laboratories either to a tedious and costly process of acquiring reference standards from various sources or to a decision of letting the quantity of NPS undetermined [3,4].

Much attention has been paid to the development of efficient identification methods for NPS in seized samples and biological material. The advent of bench-top high-resolution mass spectrometry (HR-MS) and soft ionization techniques, such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), has allowed detection of compounds based on their molecular formula by targeting the intact precursor ion with high mass accuracy. The advantage over traditional low-resolution mass spectrometry is that HR-MS enables molecular formula-based identification of drugs, which has been applied to tentative identification of NPS [5]. Molecular formula-based identification is more reliable when it involves also molecular fragmentation data [6], which can today be achieved through international collaboration, for example using a crowd-sourced online NPS database that supports multiple vendor platforms [7].

Quantitative HR-MS without reference standards is an attractive concept because of the capability to determine a vast number of compounds in a non-targeted manner. Many fields of analysis, such as forensic toxicology, environmental analysis, safety testing of pharmaceutical drug metabolites and biomarker screening would likely benefit from such an analytical approach. However, quantification without primary reference standards by MS-based techniques is not viable because ionization in MS is structure-dependent, and it is affected by external factors, such as pH and matrix interferences [8]. Hatsis *et al*. [9] explored the use of secondary reference standards in ESI-HR-MS and estimated drug metabolite concentrations from the parent drug ion responses, but they concluded that the applications were limited due to low accuracy. In their study, the response ratios for 45 metabolite-parent drug pairs ranged from 0.014 to 8.6, which signifies up to 71-fold difference in the quantitative result.

Nuclear magnetic resonance spectroscopy (NMR) is a qualified technique for both qualitative and quantitative analysis of seized materials [10,11], but this technique is not achievable for all laboratories. Recently, a new tool has emerged for the rapid quantitative estimation of NPS in blood samples using secondary reference standards [12,13]. In the latest application, Mesihää *et al*. (2019) have described a method for simultaneous detection and quantitative estimation of illicit psychostimulants in blood, based on gas chromatography nitrogen chemiluminescence detection coupled with atmospheric pressure chemical ionization quadrupole time-of-flight mass spectrometry (GC-NCD-APCI-QTOFMS) [12]. Quantitative estimation relied on the NCD's *N*-equimolar response to nitrogen, using amphetamine, 3,4-methylenedioxymethamphetamine (MDMA) and methylenedioxypyrovalerone (MDPV) as external calibrators for *prim*-, *sec*- and *tert*- amines, respectively.

There is an equally strong demand for the instant purity assessment of NPS -related seizures in the absence of primary reference standards. Consequently, in this study we extend the scope of the previously developed GC-NCD-APCI-QTOFMS method to seized samples containing stimulant-type NPS, applying a facile procedure

for sample preparation. Furthermore, we compare the quantitative results to those obtained by an established single-calibrator method relying on liquid chromatography - chemiluminescence nitrogen detection (LC-CLND).

2. Material and methods

2.1. Chemicals

Reference standards of pharmaceutical-grade purity were obtained from the following suppliers: 2C-B, 2C-T-4, 3,4-dimethoxyphenethylamine (DMPEA), 3,4-methylenedioxy-*N*-methyl-*α*-ethylphenethylamine (MBDB), 4-methylamphetamine, 4-methylthioamphetamine (4-MTA), MDDMA and mescaline were from Lipomed (Arlesheim, Switzerland). 2-Fluoroamphetamine, 4-methylethcathinone, *α*-PHP, methylone, pentedrone, PV8 and *α*-ethylaminopentiophenone were from Cayman Chemical (Ann Arbor, MI, USA). 4-Methylmethamphetamine (4-MMA), *meta*-chlorophenylpiperazine (*m*CPP) and MDMA were from National Measurement Institute (Pymble, Australia). 3-Fluorophenmetrazine, 4-fluoro-*α*-PVP, MDPV and *α*-PVP were from Chiron (Trondheim, Norway). 5,6-Methylenedioxy-2-aminoindane (MDAI), butylone, camfetamine, dibutylone, ethylphenidate and methiopropamine were from LGC GmbH (Luckenwalde, Germany). Bromo-DragonFLY was from Toronto Research Chemicals (Toronto, ON, Canada). Cocaine was from Sigma Aldrich (St Louis, MO, USA). Buspirone was from Orion Corporation (Espoo, Finland). Seized samples were received from the National Bureau of Investigation, Finland, and from the Finnish Customs Laboratory. All seized samples were assumed to exist as their hydrochloride (HCl) form.

Derivatization reagent, *N*-methyl-bis-trifluoroacetamide (MBTFA) was from Thermo Fischer Scientific (Bellefonte, PA, USA).

2.2. External calibrators

MDMA was used as an external calibrator in GC-NCD for *prim*- and *sec*- amines, and *α*-PVP for tertiary amines. In quantitative estimation by GC-NCD, a linear regression model was constructed with an appropriate external calibrator using concentrations 20, 50, 100, 200 and 500 µg/mL with three replicates. The peak area of the compounds, with two replicates each, was corrected according to the relative nitrogen content prior to applying the linear regression model. All peak areas were normalized to the peak area of the internal standard buspirone. The lowest calibration point from a 20 µg/mL working solution corresponded to 0.65 and 0.55 ng of nitrogen injected for MDMA and α-PVP, respectively.

2.3. Sample preparation

The sample preparation procedure was adapted from Meng and Margot [14], but using simple MBTFA acylation instead of dual derivatization. Briefly, 2-3 mg of the reference standards or seized powdery material was dissolved in chloroform/pyridine (5:1) to obtain a stock solution with an apparent concentration of 1000

µg/mL. The stock solution was further diluted to obtain a working solution that contained 200 µg/mL each of the 4-5 different reference standards, an external calibrator or seized material. Buspirone was dissolved in chloroform/pyridine (5:1) to obtain an internal standard solution containing 200 µg/mL of buspirone. Subsequently, 250 µL of the internal standard solution was mixed with 250 µL of the working solution. Derivatization was accomplished by adding 50 µL of MBTFA to this mixture, after which the solution was briefly mixed and heated for 30 minutes at 70℃.

2.4. GC-NCD-APCI-QTOFMS

The instrument conditions were essentially similar to those described earlier [12] with minor modifications.

The GC was operated in the split injection mode (formerly [12] in the splitless mode) at ratio of 10:1. The injector port temperature was 250°C and the transfer line temperature 320°C. The injection volume was 1.0 µL. The oven temperature was initially held at 80°C and then increased by 30°C per min to 280°C and at the rate of 10°C per min to 320°C, which was held for 4 min. Helium was used as carrier gas at 1 mL/min in the constant flow mode.

The QTOFMS was operated in the APCI positive ionization mode. Mass acquisition was performed in All Ions mode, and data were recorded over the *m/z* range of 50–450 with an acquisition rate of 5 spectra/s. Collision energy at the low energy function was 0 eV, whereas in the high energy function 22 eV was used. A mass increment of 95.9823 Da was added to the theoretical mass of each MBTFA reaction product. All data were collected and analyzed with MassHunter Data Acquisition B.04.00 and MassHunter Qualitative analysis B.07.00. software (Agilent Technologies).

2.5. LC-CLND

The reference method utilizing LC-CLND coupled with UV diode array detector for the analysis of seized NPS samples was previously described by Rasanen *et al*. [15]. In this method caffeine was used as an external calibrator.

3. Results and discussion

3.1. Peak identification

Peak identification was based on the known molecular structure and the corresponding accurate mass of the precursor ion $[M+H]^+$. Detection of the precursor ion by GC-APCI-QTOFMS scan mode was used to verify successful derivatization and to prove peak identity in subsequent quantitative GC-NCD analysis.

The acylation of stimulants by MBTFA expectedly increased the mass of the precursor ion by 95.9823 Da for each *prim*- or *sec*- amine. In GC-APCI-QTOFMS, the protonated molecule was retained with all twenty-eight

reference standards and with all forty-two NPS in seized samples. Incomplete derivatization was observed only with methoxetamine and 5-MeO-MIPT in seized samples. For methoxetamine, *m/z* 344.1468 and *m/z* 248.1645 were found, and for 5-MeO-MIPT, *m/z* 343.1628 and *m/z* 247.1805 were found, corresponding to a mixture of underivatized and derivatized product in each case. These two drugs were subsequently measured without MBTFA derivatization using the external calibrator for *tert*- amines (*α*-PVP), which resulted in a single peak with consistent shape in each case.

Simple MBTFA acylation of amino groups instead of dual derivatization was carried out here, unlike in the original study [14], because constituent profiling was not an objective. Chloroform was used as a reagent in sample preparation as per the original study, due to its indispensable chemical properties as a solvent. Appropriate caution should be taken to avoid exposure to this toxic chemical.

3.2. Equimolarity of GC-NCD using external secondary calibrators

Table 1 shows the between-day accuracy and precision of GC-NCD quantification, using secondary reference standards, for the twenty-eight pure stimulants studied at 200 µg/mL level. The grand mean equimolarity was 91.9% (CV 5.5%), as compared with primary reference standards. Table 2 shows, based on the same experimental setting, that the grand mean equimolarity over a concentration range of 40 - 200 µg/mL was 89.8% (CV 7.5%) and the bias of individual equimolarity measurements was always better than 30%, except for methylone and cocaine. There was little difference between the results across the concentration range.

Table 1. Between-day equimolarity and precision for stimulant-type NPS reference standards by GC-NCD [a].

Analyte	Mean equimolarity (%)	Median equimolarity (%)	CV (%)	Range (min-max)	
Primary amines					
External calibration with MDMA					
2C-B	88.7	89.2	6.8	79.7	95.6
2C-T-4	82.9	83.8	4.5	77.9	86.7
2-Fluoroamphetamine	81.0	81.8	4.1	76.7	84.1
4-Methylamphetamine	99.3	99.5	5.3	90.8	104.8
4-Methylthioamphetamine	91.0	87.1	7.1	85.4	99.6
Bromo-DragonFLY	82.6	83.6	4.1	76.8	85.6
DMPEA	88.2	88.0	5.7	81.0	94.6
Primary amines mean	**87.7**	**87.6**	**5.4**		
Secondary amines					
External calibration with MDMA					
3-Fluorophenmetrazine	80.0	79.3	6.7	74.9	88.7
4-Methylethcathinone	84.6	83.4	4.4	80.0	89.4

4-Methylmethamphetamine	92.9	93.0	2.1	90.7	95.5
Butylone	88.4	84.5	9.8	83.3	103.7
Camfetamine	85.0	86.3	6.9	75.5	89.8
Ethylphenidate	74.8	74.9	3.2	71.0	77.5
MBDB	93.9	94.3	5.0	86.2	98.5
mCPP	97.4	97.7	3.7	92.1	101.2
MDAI	83.2	82.4	2.8	80.7	86.2
Mescaline	92.9	93.4	3.8	88.0	97.8
Methiopropamine	94.8	96.2	6.0	85.8	101.3
Methylone	73.0	70.8	6.4	69.8	81.0
Pentedrone	83.6	82.7	6.5	77.1	89.2
α-Ethylaminopentiophenone	97.9	96.3	5.7	91.6	105.3
Secondary amines mean	**87.3**	**86.8**	**5.2**		
Tertiary amines					
External calibration with α-PVP					
4-Fluoro-α-PVP	108.9	108.9	2.8	105.0	113.2
Cocaine	136.2	136.7	1.3	133.8	138.3
Dibutylone	106.0	108.8	8.9	94.2	117.3
MDDMA	107.1	109.7	8.5	96.5	116.8
MDPV	92.0	89.9	8.3	84.0	104.4
PV8	91.0	87.9	7.6	84.4	102.2
α-PHP	95.3	94.8	5.6	88.8	102.1
Tertiary amines mean	**105.2**	**105.2**	**6.1**		
Grand mean	**91.9**	**91.6**	**5.5**		

[a] Data represents mean values from five separate experiments, each measured in duplicate

Table 2. Equimolarity and precision for stimulant-type NPS reference standards by GC-NCD at five concentration levels [a].

Analyte	Equimolarity (%) at specific sample concentration (µg/mL)						
	40	80	120	160	200	Mean	CV (%)
Primary amines							
External calibration with MDMA							
2C-B	83.3	75.3	90.1	85.9	83.0	83.5	11.2
2C-T-4	79.2	76.1	78.5	71.4	81.9	77.4	7.6
2-Fluoroamphetamine	87.0	85.7	84.6	82.4	82.8	84.5	6.2
4-Methylamphetamine	80.5	100.2	101.3	105.8	98.7	97.3	10.8
4-Methylthioamphetamine	102.8	93.5	90.4	87.4	90.1	92.8	8.2
Bromo-DragonFLY	73.2	72.1	85.3	78.7	75.5	77.0	7.7
DMPEA	99.6	91.2	92.0	91.4	86.9	92.2	7.7
Primary amines mean	**86.5**	**84.9**	**88.9**	**86.1**	**85.6**	**86.4**	**8.5**
Secondary amines							

External calibration with MDMA							
3-Fluorophenmetrazine	80.6	76.4	81.9	78.5	75.4	78.6	6.1
4-Methylethcathinone	84.0	89.3	88.2	87.3	88.3	87.4	6.4
4-Methylmethamphetamine	96.2	96.9	100.1	99.3	100.4	98.6	4.8
Butylone	99.7	88.5	86.7	85.6	86.5	89.4	9.6
Camfetamine	93.6	85.0	83.3	79.5	86.3	85.5	7.4
Ethylphenidate	90.5	79.5	78.9	75.1	75.3	79.9	7.9
MBDB	102.1	93.5	94.2	89.6	89.6	93.8	7.5
mCPP	103.7	81.4	93.7	95.2	97.4	94.3	9.2
MDAI	75.5	78.6	77.4	78.6	83.1	78.7	3.2
Mescaline	100.0	95.8	98.2	99.3	97.6	98.2	7.5
Methiopropamine	87.0	83.4	93.2	93.0	91.3	89.6	6.0
Methylone	66.8	61.0	66.9	66.3	66.5	65.5	5.5
Pentedrone	74.5	82.7	79.9	75.8	77.6	78.1	5.3
α-Ethylaminopentiophenone	114.2	104.2	106.9	101.4	111.9	107.7	5.4
Secondary amines mean	**90.6**	**85.4**	**87.8**	**86.0**	**87.7**	**87.5**	**6.6**
Tertiary amines	-	-	-	-	-	-	-
External calibration with α-PVP							
4-Fluoro-α-PVP	98.0	95.6	99.9	101.6	103.8	99.8	5.2
Cocaine	118.2	145.0	139.2	133.3	139.3	135.0	8.3
Dibutylone	104.5	106.4	108.8	110.7	110.3	108.2	10.5
MDDMA	97.5	103.8	107.6	106.8	107.6	104.6	5.3
MDPV	76.7	74.3	73.4	86.3	86.4	79.4	9.0
PV8	71.1	68.7	76.3	80.2	83.3	75.9	10.8
α-PHP	80.3	76.4	82.3	81.5	87.1	81.5	9.0
Tertiary amines mean	**92.3**	**95.7**	**98.2**	**100.1**	**102.5**	**97.8**	**8.3**
Grand mean	**90.0**	**87.9**	**90.7**	**89.6**	**90.9**	**89.8**	**7.5**

[a] Data represents mean values of two separate measurements per each concentration

Contrary to MS or UV detection, the *N*-equimolar response of NCD enables a uniform response to nitrogen-containing compounds regardless of analyte structure. It was shown by Yan *et al*. [16] that approximately 15-20% variation in equimolarity could be expected when analyzing structurally different organic nitrogen compounds, while for compounds with adjacent nitrogen atoms the signal was considerably quenched. Basically, only one nitrogen-containing external calibrator is sufficient for universal calibration by NCD, but more calibrators help control the sample preparation stage.

3.3. Purity estimation of seized material

The GC-NCD-APCI-QTOFMS method was used to analyze forty-two seized powdery samples previously found to contain stimulant-type NPS. The drugs were identified by HR-MS and, initially by NMR spectroscopy, prior to submitting the samples for quantitative estimation. An established single-calibrator LC-CLND method [15] was used to provide a reference value for the comparison of purity values. Table 3 shows

that the grand mean absolute difference between the purity values from the two methods was 8.1% and the range was 0.4 - 26.7%. The absolute difference was > 20% in only two cases (brephedrone and MDPBP).

Table 3. Comparison of GC-NCD method to reference LC-CLND method for purity estimation of stimulant-type NPS in seized material [a].

Analyte	Purity % (GC-NCD)	Purity % (LC-CLND)	Difference (%)
Primary amines			
External calibration with MDMA			
2C-C	83.7	89.1	5.4
2C-T-7	90.4	83.3	7.1
4-Fluoroamphetamine	82.7	81.0	1.7
Allylescaline	81.3	85.4	4.1
DOC	92.7	97.8	5.1
MDAI	59.6	76.4	16.8
Secondary amines			
External calibration with MDMA			
3,4-CTMP	94.4	90.3	4.1
3,4-DMMC	70.1	82.4	12.3
4-CEC	84.0	87.4	3.4
4-Ethylmethcathinone	87.1	86.6	0.5
4-Fluoroethylphenidate	96.3	96.7	0.4
4-Fluoromethylphenidate	98.3	86.3	12.0
4-MEAP	94.8	92.8	2.0
4-Methylbuphedrone	87.8	75.2	12.6
5-EAPB	94.8	93.3	1.5
5-MeO-MIPT	62.1 [b]	78.9	16.8
Brephedrone	70.6	94.4	23.8
Buphedrone	87.3	71.2	16.1
Clephedrone	74.1	84.5	10.4
Ethylone	37.4	30.2	7.2
HDMP-28	92.5	94.8	2.3
Mephtetramine	73.3	63.7	9.6
Methiopropamine	94.2	81.2	13.0
Methoxetamine	58.5 [b]	71.2	12.7
Methylone	92.8	85.5	7.3
N-Ethylhexedrone	99.8	90.8	9.0
N-Ethylpentedrone	101.2	104.1	2.9
N-Ethylpentylone	101.8	101.4	0.4
Pentedrone	104.3	88.4	15.9
Thiothinone	91.5	74.2	17.3
threo-4-Methylmethylphenidate	93.1	91.2	1.9
Tertiary amines			
External calibration with α-PVP			
4-Fluoro-PV-9	75.1	87.4	12.3
4-Fluoro-α-PHiP	35.0	39.6	4.6

4-Fluoro-α-PVP	98.2	97.8	0.4
Cocaine	37.5	25.3	12.2
MDPBP	61.5	88.2	26.7
MDPV	21.6 [c]	22.9	1.3
Pyrovalerone	81.9	83.3	1.4
α-PHiP	104.2	87.5	16.7
α-PPP	76.0	82.1	6.1
α-PVP	77.9	75.9	2.0
α-PVT	100.3	101.2	0.9
Grand mean	**81.0**	**81.0**	**8.1**

[a] Purity is reported for hydrochloride (HCl) salt as an average of two separate samplings measured in duplicate

[b] Sample was re-measured without MBTFA using α-PVP as external calibrator due to incomplete derivatization

[c] Sample was re-measured using 1000 µg/mL working solution instead of 200 µg/mL to obtain well-resolved peak

3.4. Advantages and limitations

There are certainly alternative detectors to NCD available, which exhibit a fairly uniform response for quantification. These detectors, such as charged aerosol detector (CAD), evaporative light scattering detector (ELSD) and vacuum ultraviolet detector (VUV) were comprehensively reviewed by Zhang *et al*. 2019 [17]. The clear advantage of NCD over the universal detectors is its high selectivity to drugs, as only nitrogenous compounds are detected, and to top it all, this takes place in an equimolar manner. These features have allowed analysis of complex matrices, such as blood or urine, with a sufficiently high signal to noise ratio [12,18]. Some drugs do not contain nitrogen, and hence another technique, such as LC-CAD [19] should be considered.

The developed GC-NCD-APCI-QTOFMS method enables peak identification followed by quantitative estimation of stimulant-type NPS at an acceptable level of accuracy at least for a preliminary analysis. GC –based methods are generally less amenable to quantitative analysis of polar compounds than LC –based methods. Thus the performance, in terms of equimolarity obtained by the present method for stimulants, was slightly less (91.9%) than what was reported in a previous paper (94.4%) by LC-CLND [15]. However, GC-NCD-APCI-QTOFMS allows simultaneous peak identification with high chromatographic and mass resolution [20], which is superior to the peak purity algorithm of the UV diode array detector in the LC-CLND platform. Hence NCD-APCI-QTOFMS is capable of identifying other co-eluting constituents or impurities that could interfere with the quantification. Unfortunately, the manufacturer of CLND instrumentation has informed about discontinuation of the production of the LC version of the instrument.

Comparison of the present method to another method that shares the same principle for detection and external secondary calibration is a limitation (NCD and CLND are abbreviations given by the respective instrument manufacturers). However, our decision to choose LC-CLND as a reference method was based on the fact that neither certified primary reference standards nor validated conventional reference methods for all the NPS found in the seized samples were available to the authors.

Apart from NMR spectroscopy [11], there has been limited research activity within forensic sciences concerning identification combined with universal quantification methods in the absence of primary reference standards. However, with MS and UV –based methods, relying only on secondary standards is risky due to compound-specific ion responses [9,21]. Importantly, there are no established guidelines for analysis in such a scenario where the primary reference standards are missing, however, we think that implementation of a statistical uncertainty model could be beneficial in such circumstances [21].

4. Conclusions

Lack of primary reference standards for NPS prevents their appropriate identification and quantitative purity determination by using conventional techniques. We have developed and tentatively validated a GC-NCD-APCI-QTOFMS method for the quantitative estimation of stimulant-type NPS applied to seized powdery material, using secondary reference standards for calibration. The presented method allowed purity estimation with a high average accuracy. Instant quantitative assessment of seized NPS, with the option to analyze their impurity profiles, creates valuable opportunities for quick response. However, as established forensic guidelines for analysis based only on secondary calibrators are missing, the present method serves predominantly as a rapid test producing the grounds for possible further measures. In any case, we anticipate that the present approach will find further applications to other classes of NPS as well as in other fields of forensic analysis.

CRediT authorship contribution statement

Samuel Mesihää: Conceptualization, Formal analysis, Methodology, Writing – original draft. **Ilpo Rasanen**: Methodology. **Ilkka Ojanperä:** Conceptualization, Supervision, Writing – review & editing.

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