Microchemical Journal 213 (2025) 113770

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Determination of ketamine and norketamine in hair samples by μ-QuEChERS and gas chromatography coupled to tandem mass spectrometry

Rodrigo Pelixo [a,b,c], Mário Barroso [d,e], Tiago Rosado [a,b,c,f], Eugenia Gallardo [a,b,c,f,*]

[a] Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior (CICS-UBI), 6200-506 Covilhã, Portugal
[b] Laboratório de Fármaco-Toxicologia, UBIMedical, Universidade da Beira Interior, EM506, 6200-000 Covilhã, Portugal
[c] RISE-Health, Departamento de Ciências Médicas, Faculdade de Ciências da Saúde, Universidade da Beira Interior, 6200-506 Covilhã, Portugal
[d] AlphaBiolabs, 14 Webster Court, Carina Park, Warrington WA5 8WD, UK
[e] Serviço de Química e Toxicologia Forenses, Instituto Nacional de Medicina Legal e Ciências Forenses-Delegação do Sul, 1169-201 Lisboa, Portugal
[f] Centro Académico Clínico das Beiras (CACB) - Grupo de Problemas Relacionados com Toxicofilias, 6200-000 Covilhã, Portugal

ARTICLE INFO

Keywords:
QuEChERS
Hair
Ketamine
Norketamine

ABSTRACT

Ketamine has gained popularity in Europe as a new psychoactive substance due to its dissociative effects, which are sought after by users at raves, for unprescribed treatment of psychological issues, and in drug-facilitated crimes. Forensic experts often face challenges in proving drug use, and hair offers distinct advantages as a sample, revealing both long-term consumption and recent abuse. However, due to its complex nature, hair requires pretreatment to remove interferences and enhance analytical sensitivity.

This study employs a miniaturised version of the "Quick, Easy, Cheap, Rugged, and Safe" (QuEChERS) extraction method, commonly used in food and environmental analyses. The method uses 1 mL of 5 % formic acid in acetonitrile and 250 mg ammonium formate as solvent and salt partitioning agents, along with 50 mg magnesium sulphate and 10 mg primary-secondary amine for dispersive solid-phase extraction. After this clean-up process, samples were analysed using gas chromatography coupled with tandem mass spectrometry.

Following optimisation, the method was validated for detecting ketamine and its primary metabolite, norketamine. A limit of detection of 0.01 ng/mg was achieved for ketamine, and 0.8 ng/mg for norketamine, with limits of quantification of 0.05 ng/mg and 0.8 ng/mg, respectively. This established a working range of 0.05–5 ng/mg for ketamine and 0.8–5 ng/mg for norketamine. Recovery rates ranged from 47–76 % for ketamine and 14–27 % for norketamine.

The method was then applied to real samples from drug users, proving the effectiveness of the μ-QuEChERS technique as a sustainable clean-up method. Despite its miniaturisation, it retained the performance of the original QuEChERS protocol and allowed obtaining high sensitivity, enabling analyte detection at a concentration below the Society of Hair Testing's cut-off value of 0.2 ng/mg.

1. Introduction

Arylcyclohexylamines are considered a drug group within new psychoactive drugs by the European Union Drug Agency, anchored on the synthesis of phencyclidine (PCP) in 1956 as analgesic by Parke-Davis which was later considered a schedule II in the Controlled Substances Act of the Drug Enforcement Administration (DEA) [1]. After the adverse psychological effects caused by the multiple interactions in the Central Nervous System (CNS) [2,3], it was discontinued and a new compound named CI-581, now known as ketamine (KET), began clinical trials as a safer substitute for PCP[4]. KET possesses less side effects compared to the predecessor, creating a state of dissociative anaesthesia without the delirium caused by PCP and had a shorter half-life [5,6] but practitioners remained cautious about post-operative dissociative

* Corresponding author at: Centro de Investigação em Ciências da Saúde, Universidade da Beira Interior (CICS-UBI), 6200-506 Covilhã, Portugal.
E-mail addresses: pelixo.silva@ubi.pt (R. Pelixo), mbarroso@alphabiolabs.com (M. Barroso), tiago.rosado@ubi.pt (T. Rosado), egallardo@fcsaude.ubi.pt (E. Gallardo).

https://doi.org/10.1016/j.microc.2025.113770
Received 18 January 2025; Received in revised form 6 April 2025; Accepted 24 April 2025
Available online 24 April 2025

permanence leading to initial decrease in medicinal utilization, which nowadays has been restored due to better knowledge and substance control [7,8]. Nonetheless, KET became a sought-after substance in illicit markets precisely due to its psychotomimetic effects, being considered one of the pioneer new psychoactive substances in Europe, and now a schedule III-controlled substance in America [9,10]. These side-effects are caused by the non-competitive interaction with the PCP binding site of the NMDA receptors, leading to an allosteric transformation blocking the ion transport that these channels are responsible for, leading to the characteristic dissociative state, confusion and euphoria caused by the drug [5,11]. Hence consumers range from hospital and veterinary workers and associates with access to the pharmaceutical compound, to rave and electronic dance parties' goers, creating a large dissemination through society due to the movement expansion [6,8,10]. The consumer trend requires that forensic analysts focus on this drug, since it imposes a threat to society due to the dissociative state, euphoria and confusion it induces, leading to misfortunate events like violent crimes, sexual assaults, medical malpractice, impaired driving and other incidents [12–14].

A forensic specialist thrives due to the wide array of methodologies and constant improvement in the analysis applied to biological matrices. Besides the classical matrices like blood and derivates and urine, alternative matrices can be advantageous and thus have gained a spotlight in recent years. From these, hair has emerged as a trend as it allows differentiating chronical from single use and provides information on consumption patterns via segmental analysis. However, hair has disadvantages, being one of them its inherent complexity, often requiring clean-up in the sample pre-treatment process [15–18]. Analysts have developed diversified strategies for these analytes' extraction and detection in hair samples, aiming at detecting low concentrations and enabling identifying drug users by application of cut-off levels (0.2 ng of KET per mg of hair) [18]. Different approaches have been employed, from solid-liquid extraction with different solvents [19,20], coupling of other techniques like microwave assisted extraction [21], or cryogenic grinding [22] and solid-phase extraction (SPE) [23,24]. These methodologies often allow obtaining high sensitivity with good recoveries, however with poor performance in other aspects, for instance by using hazardous reagents, equipment that increase the complexity of sample treatment and high amounts of expensive and discardable products. In this context, is noticeable a gap in laboratories of routine analysis for workflow approaches that can achieve considerable results, whilst keeping the cost per analysis low, with minimal production of residues and waste.

The Quick Easy Cheap Rugged and Safe (QuEChERS) approach is an extraction and clean-up technique devised for pesticide residues in the food and environmental fields. The technique is composed of two major steps, a partition based on salting-out and a dispersive solid-phase extraction, resulting in a cost-effective, versatile and easy to apply method with overall diminished constrains in the workflow [25,26]. Besides these, QuEChERS represents a sample treatment procedure that is commonly employed in analysis routine laboratories, since it is the official method for pesticide residues in Europe (European Standard EN 15662) and the USA (AOAC 2007.01 [27]). The method also complies with several principles of green chemistry [28], as it commonly uses fewer toxic reagents and allows miniaturisation [29].

Due to these characteristics, the extraction technique and its variations have expanded into other areas, including forensic analysis [30–33] and in specific hair [34–36]. This application comes to fruition from technique effect on a broad range of interferents by conjugating diverse chemical, physical and mechanical interventions on the sample, affecting not only the macromolecules of its composition, but also other contaminants that have the capacity to impregnate hair due to the natural exposure of the matrix. This association between the clean-up capacity of the technique and the nature of hair, as well as the gap beforementioned in routine hair testing, calls for the application of this type of approach to the analysis of KET in hair.

The aim of this study was to develop a QuEChERS clean-up method for determining KET and its primary metabolite, norketamine (NKET), in hair, using gas chromatography coupled with tandem mass spectrometry for analysis.

2. Materials and methods

2.1. Reagents and standards

The analytical standards for KET and NKET, along with the deuterated internal standard (IS) NKET-d4, were obtained from LGC Promochem (Barcelona, Spain). Acetic acid, methanol, dichloromethane, and acetonitrile were acquired from Fisher Scientific (Loughborough, UK). Potassium dihydrogen phosphate and dipotassium phosphate were purchased from Enzymatic (Santo Antão do Tojal, Portugal). Formic acid and ammonium acetate were sourced from Sigma-Aldrich (Sintra, Portugal), while the primary secondary amine (PSA), magnesium sulphate, sodium acetate, and ammonium formate were obtained from Laborspirit (Sintra, Portugal). Deionised water was supplied by a Milli-Q system (Millipore, Billerica, MA, USA).

The standard stock solutions had a concentration of 100 μg/mL. Working solutions were prepared by diluting these to concentrations of 100 ng/mL, 1 μg/mL, and 10 μg/mL in methanol, while the IS solution was prepared in the same way, diluting the 100 μg/mL stock to 5 μg/mL.

2.2. Hair samples

The experimental procedure required blank hair samples for optimisation and validation, which were collected from laboratory staff at the Health Sciences Research Centre (Covilhã, Portugal) and acquaintances who had not been exposed to KET in any form. Real hair samples were obtained from drug users at an electronic music festival held between the 22nd and 29th of July 2022. Collection was performed in accordance with the Declaration of Helsinki, and the study was approved by the Ethics Committee of Universidade da Beira Interior (reference CE-UBI-Pj-2022–04).

All samples were collected from the vertex posterior, as close to the scalp as possible, using decontaminated scissors. The samples were stored in paper envelopes at ambient temperature, protected from direct sunlight, and properly labelled (without any identification of the sample donor).

2.3. Chromatographic and mass spectrometric conditions

An Agilent Technologies HP 7890A GC system coupled to an Agilent Technologies 7000B QqQ MS (Waldbronn, Germany) was selected for the chromatographic analysis. A capillary column with a length of 30 m, an internal diameter of 0.25 mm, and a film thickness of 0.25 μm, consisting of (5 %-phenyl)-methylpolysiloxane from Agilent J&W (Santa Clara, CA, USA) was used. The initial temperature of 150 °C was held for 0.5 min before increasing 5 °C per minute to a final temperature of 280 °C, which was maintained for 1 min, resulting in a total run time of 27.5 min. The inlet temperature was set at 250 °C, and 2 μL of the sample was injected in splitless mode, with a helium carrier gas flow of 0.8 mL/min. The source and detector temperatures were maintained at 230 °C and 250 °C, respectively, while in the collision cell, the helium and nitrogen flows were set to 1.5 mL/min and 2.5 mL/min, respectively. Electron ionisation mode was used, with a current of 35 μA and an energy of 70 eV. Data analysis was performed using MassHunter Workstation software version B.02.01 from Agilent Technologies.

The transitions, retention times, collision energies, and dwell times are detailed in Table 1.

2.4. Sample preparation

The sample preparation procedure began with hair decontamination

Table 1
MS detection parameters.

Analyte	Transitions (*m/z*)	Retention time (minutes)	Collision Energy (eV)	Dwell time (ms)
NKET-d4	169.2 –> 134.0	10.09	20	50
KET	181.0 –> 116.1*	10.69	20	50
	181.0 –> 151.0	10.69	10	50
NKET	194.4 –> 131.0*	10.09	20	50
	194.4 –> 166.0	10.09	20	50

*Quantifying transition

by consecutively washing the samples in dichloromethane, deionised water and methanol for 15 min each on a tube roller. After each wash, the solvent was discarded, and the samples were dried with paper. Following the final wash, the hair was left to dry overnight.

Once dried, the hair was cut into small segments, and 50 mg of the sample was weighed into a glass tube. To this, 2 mL of methanol was added. The mixture was homogenised using a vortex mixer and then heated to 65 °C overnight. The supernatant was transferred to a new glass tube, and 10 μL of IS at a concentration of 5 μg/mL was added. The solvent was then evaporated under a nitrogen stream. Finally, the extract was reconstituted in 1 mL of 0.1 M phosphate buffer at pH 5.5 before proceeding with the clean-up procedure.

2.5. QuEChERS optimisation

To optimise the performance of the QuEChERS method, the partitioning efficiency of various salts and solvents was evaluated using Friedman's Two-Way Analysis of Variance for Related Samples in SPSS Statistics. Subsequently, a Design of Experiments (DOE) approach was employed in Minitab version 17 to refine the quantities of salts and solvents. The selected variables included solvent volume, salting-out salt quantity, d-SPE salt quantity, and d-SPE sorbent quantity.

The final μ-QuEChERS protocol was as follows: 1 mL of the reconstituted sample in phosphate buffer was mixed with 250 mg of ammonium formate and 1 mL of 5 % formic acid in acetonitrile. The mixture was agitated using a vortex mixer for 10 s, followed by centrifugation at 3500 rpm for 2 min. The resulting supernatant was collected, and 50 mg of magnesium sulphate along with 10 mg of PSA was added. After repeating the agitation and centrifugation steps, the supernatant was collected again and evaporated under a nitrogen stream. Finally, the extract was reconstituted in 30 μL of methanol and injected into the gas chromatographic system.

3. Results and discussion

3.1. QuEChERS clean-up method development and optimisation

The QuEChERS method relies on the interactions between partitioning components—solvents, salts, and sorbents—and their interaction with the sample matrix to effectively extract the analyte [37,38]. Consequently, the development of this technique requires a systematic approach, beginning with an evaluation of each component's capacity to remove interferents, followed by the selection of the most suitable materials. Finally, the optimisation of component quantities is essential to enhance extraction efficiency.

3.1.1. QuEChERS partition components selection

As a starting point, a previously validated QuEChERS method developed in the laboratory [39] was employed, adapted and miniaturised from the AOAC 2007.01 official method for pesticides [27]. This method was applied following the complete hair preparation procedure described in Section 2.4 and served as the foundation for developing the clean-up step of hair extracts fortified with KET. Modifications were required to tailor this protocol to the analysis of KET in hair using gas chromatography-tandem mass spectrometry (GC–MS/MS). Key differences from the AOAC official method included the sample amount, salt quantities in the salting-out step (while maintaining their ratios), adjustments to d-SPE salt quantities, the use of vortex mixing, and centrifugation parameters. Although previously validated for other analytes in different matrices and for liquid chromatography, the method required optimisation to address the specific characteristics of KET in hair.

To guide this optimisation, a literature review was conducted to identify appropriate partitioning components [26,29,36,40]. The study was divided into two stages: (1) evaluation of the clean-up capacity of different salts in the salting-out step, and (2) evaluating the same characteristic for different salting-out solvents. The choice of d-SPE components is discussed at the end of this section.

Initially, salts were selected based on their capacity for efficient phase separation with acetonitrile, a solvent pre-emptively chosen to integrate the organic layer during partitioning derived from its capacity of separation from the aqueous layer [25]. From literature, a mixture of magnesium sulphate ($MgSO_4$) and sodium acetate (NaOAc), and ammonium acetate (NH_4OAc) individually, were chosen for their established performance in QuEChERS protocols. Additionally, ammonium formate (NH_4HCO_2) was included due to its high volatility, which reduces interferences in analysis and improves chromatography [40]. To evaluate these salts' partitioning capacity, three experiments were conducted using the initial protocol, varying the salts as follows: (1) 0.4 g $MgSO_4$ with 0.1 g NaOAc, (2) 0.5 g NH_4OAc, and (3) 0.5 g NH_4HCO_2. Each experiment was performed in triplicate, and the mean abundances were statistically analysed using Friedman's test in SPSS Statistics software, a non-parametric test for pairwise comparison for the significance of the response obtained from each experiment. The analysis indicated a significant difference between NH_4OAc and NH_4HCO_2, while the $MgSO_4$ and NaOAc combination was deemed unsuitable, since it was obtained a significance level of 0.662 for both pairwise comparisons of the combination for an established level of 0.05 (Supplementary material Fig. S1). NH_4HCO_2 was ultimately selected for its superior volatility, which minimised equipment contamination, improved overall analyte recovery and whilst achieving higher relative abundance (Supplementary material Fig. S2). In contrast, $MgSO_4$ was noted to cause deposition in the GC liner, negatively impacting analysis due to its low volatility [26].

For the solvent study, acetonitrile was the primary candidate due to its proven compatibility with aqueous solutions, as previously mentioned. Alternative solvents found in literature, such as ethyl acetate, dichloromethane, and hexane were excluded to prioritise the method's environmental sustainability. Methanol, while a viable option for dissolving KET and its metabolite NKET, was not considered due to challenges in phase separation. Acidification of the solvent is a common strategy for alkaline analytes like KET [26]; therefore, in the same style as the salts experiment, three solvents were tested: (1) acetonitrile, (2) 1 % acetic acid in acetonitrile, and (3) 5 % formic acid in acetonitrile. The statistical analysis revealed a significant difference between 1 % acetic acid in acetonitrile and 5 % formic acid in acetonitrile (Supplementary material Fig. S3), with the latter yielding higher relative abundance (Supplementary material Fig. S4). Thus, 5 % formic acid in acetonitrile was selected as the extraction solvent.

In the d-SPE step, primary-secondary amine (PSA), octadecyl silica (C_{18}), and graphitised carbon black (GCB) were considered based on the literature. GCB is primarily used for samples with high pigment content, such as carotenoids and chlorophyll, while C_{18} is effective for lipid-rich samples. PSA was selected for its broad-spectrum activity, capable of removing sugars, lipids, organic acids, fatty acids, and certain pigments [26]. Although $MgSO_4$ was associated with low volatility issues, its

ability to reduce water content made it a valuable component in the d-SPE step, particularly given the smaller quantities required compared to the salting-out step [25].

3.1.2. QuEChERS optimisation with DOE factorial design

Once the solvents and reagents were selected, the quantities of each constituent were optimised. To achieve this, a DOE approach was employed, utilising a 2-level full factorial design. The parameters and their respective low and high limits were established based on previous literature (Table 2).

The experiments, designed by the software to assess the interaction of multiple factors, were executed accordingly, with the respective responses recorded. Analysis of the Pareto chart (Fig. 1) revealed no significant variation in the clean-up procedure. From the main effects plot, it was observed that the solvent volume was the most influential factor, with a higher solvent volume resulting in a greater response. A similar trend was noted for the amount of NH_4HCO_2, while the quantities of PSA and $MgSO_4$ exhibited an opposite effect (Fig. 2). The interaction plot further indicated that no factor significantly influenced the others within the limits stablished for the DOE (Fig. 3).

These results would typically allow for the selection of conditions yielding the optimal clean-up procedure; however, the lack of significance suggests that, within the set limits, the quantities of the individual components, and their interactions, do not play a substantial role. Consequently, the decision was made to adopt the most efficient method, using the lowest limit values for all factors for the remainder of the study.

It is important to note that the results presented above pertain to a second DOE experiment. During the first experiment, it became apparent that handling a larger number of samples, along with the high RPM applied during the centrifugation steps, caused the glass tubes to collapse. Since phase separation was efficiently achieved in both steps, the decision was made to reduce both the centrifugation speed and duration to 3500 RPM for 2 min, which the tubes could withstand for the remainder of the study.

Hence, the final μ-QuEChERS protocol consists of the addition of 0.25 g of NH_4HCO_2 and 1 mL of 5 % formic acid to the reconstituted sample prior to agitation in the vortex mixer and centrifugation as previously mentioned. After collection of the organic layer, 0.05g of $MgSO_4$ and 0.01g of PSA were added and after new agitation and centrifugation, the supernatant was evaporated under nitrogen stream, and finally reconstituted in 30 μL of methanol for chromatographic analysis.

3.2. Validation

The validation of the analytical method was carried out taking into consideration the guidelines provided by the Society of Hair Testing (SoHT) for drug testing in hair [18] and following the ANSI/ASB Standard 036 (1st edition) — Standard Practices for Method Validation in Forensic Toxicology [41]. The validation process involved assessing several parameters, including selectivity, linearity, limits, precision (intra-day, inter-day, and intermediate), accuracy, and recovery.

3.2.1. Selectivity

Selectivity refers to the ability of a method to determine the analyte in isolation from any interferents originating from the matrix or its pre-treatment. According to established guidelines, selectivity was assessed using blank samples from ten different origins without the addition of the IS, to demonstrate the absence of interferents at the relevant retention times and transitions. Additionally, a pooled sample containing the IS and a mixture of KET and NKET at the lower limit of quantification (LLOQ) was included for comparison. The results showed no significant interferents at the retention times or in the transitions of either ion. Consequently, the method was deemed selective for both analytes.

Table 2
Parameters and limits for the factorial design.

Factor	Low	High
5 % formic acid in acetonitrile volume (mL)	1	5
NH_4HCO_2 amount (g)	0.250	1
MgSO4 amount (g)	0.050	0.3
PSA amount (g)	0.010	0.1

3.2.2. Linearity and limits

To assess linearity, a pool of the ten blank samples previously examined for selectivity was fortified to establish a working range, which included six calibrators for each analyte. The working range for KET was 0.05–5 ng/mg, and for NKET, it was 0.8–5 ng/mg. These working ranges were evaluated over a five-day period to establish a mathematical correlation between the analyte's-to-IS peak area ratio and analyte concentration. Weighted linear regression was used to construct the model, addressing the heterocedasticity of the wide working ranges. The criteria for a valid calibration model include an R2 of at least 0.99 and a bias not exceeding 20 % for any calibrator. Table 3 summarizes the results obtained for linearity and limits.

The working range represents the concentration levels that the laboratory typically expects to encounter in routine analysis. In this study, the working ranges for both compounds are wide (one and two orders of magnitude for KET and NKET, respectively), offering a versatile range capable of detecting both chronic and occasional KET users. Furthermore, for KET, the cut-off value of 0.2 ng/mg, as stipulated by the SOHT guidelines, is well within this range, allowing for the effective identification of drug users [18]. Although the linearity for NKET did not obtain the same results as those of KET, the guidelines clearly state that no cut-off value has been established for the metabolite. The identification of NKET, regardless of its concentration, is sufficient to confirm KET consumption.

The LLOQ is defined as the lowest concentration that can be quantified with acceptable precision and accuracy. It corresponds to the lowest non-zero calibrator within the working range. In this study, the LLOQ values were 0.05 ng/mg for KET and 0.8 ng/mg for NKET (Fig. 4).

The limit of detection (LOD) is determined as the lowest concentration at which the analyte can be reliably detected, where the peak is distinguishable from the background noise. The LOD is typically defined by a signal-to-noise ratio of at least 3.3, with reproducibility established through duplicate measurements over three independent runs. This approach was applied to determine the LOD for KET, which was 0.01 ng/mg. For NKET, the LOD was the same as the LLOQ, 0.8 ng/mg.

It is worth noting that, despite the variety of approaches developed for hair analysis, none of the previously reported methods employed QuEChERS as clean-up technique. This highlights the novelty of the method developed in this study, which integrates QuEChERS for clean-up. Chang et al. [21] obtained a LOD and lower limit of quantitation (LLOQ) of 0.0005 ng/mg and 0.002 ng/mg, respectively, using microwave-assisted extraction with 10 mg of sample in methanol and TFA, followed by LC-MS/MS for detection. Although this approach yielded higher sensitivity with a smaller sample size and without a clean-up step, the use of a strong acid was required to achieve these results.

Additionally, Barreto et al. [24] employed an online SPE system coupled with LC-MS/MS after phosphate buffer extraction, achieving a LOD of 0.008 ng/mg and 0.015 ng/mg, and a LOQ of 0.02 ng/mg and 0.04 ng/mg for KET and NKET, respectively, with 30 mg of hair. SPE is a commonly used solution for extraction and clean-up but has the disadvantage of employing single-use cartridges.

Rhee et al. [23] developed another extraction method, using a mixture of methanol, acetonitrile, and ammonium formate, without incorporating a clean-up step. Using just 2.5 mg of sample, the method achieved a LOQ of 0.1 ng/mg and a LOD of 0.02 ng/mg for both

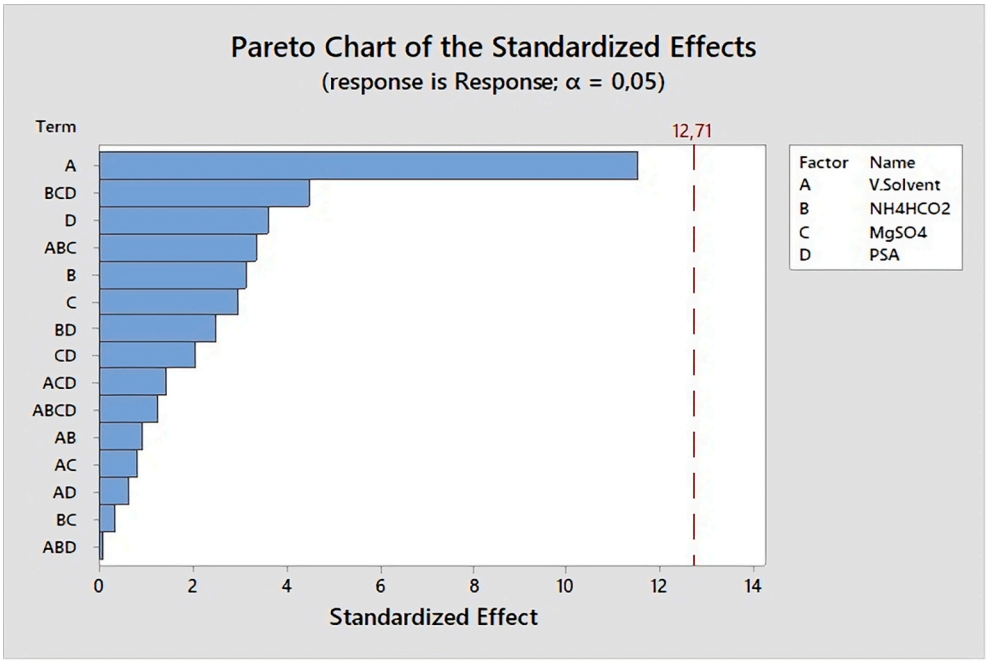

Fig. 1. Pareto chart of the standardized effects.

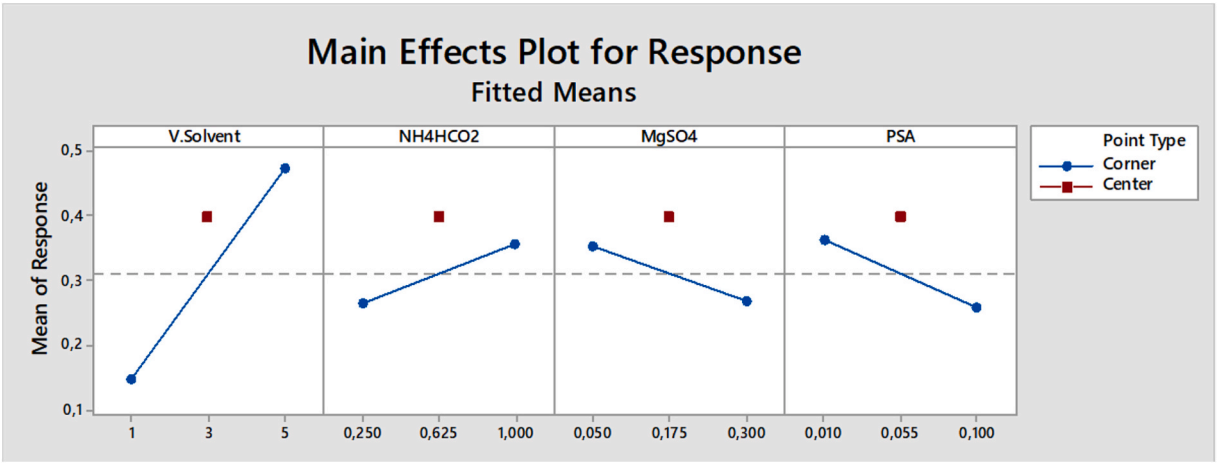

Fig. 2. Main effects plot for response.

analytes.

3.2.3. *Precision and accuracy*

A trustworthy analysis is one that can measure concentrations repeatedly, obtaining results that are close to the true value with minimal dispersion between measurements. This reliability is characterised by bias, which refers to the proximity between the mean of the measurements and the actual value (often termed relative error), and precision, which indicates the variability across multiple measurements. When both bias and precision criteria are met, the method can be considered precise and accurate.

Blank samples were spiked with four concentrations per analyte and tested over a five-day protocol to evaluate low, medium, and high concentrations within the working range. The acceptance criteria for both parameters require that all values fall below 20 %.

Intraday precision and bias evaluate the deviation of results and their proximity to the nominal value within a single run, using triplicates for low, medium, and high concentrations over five days. The studied concentrations were 0.05, 0.2, 1 and 5 ng/mg for KET and 0.8, 1, 3 and 5 ng/mg for NKET.

In this study, the cut-off value was also used as a quality control. Interday precision and bias assess the same characteristics across different runs over five days, using the same concentration levels. The intermediate precision represents a comprehensive evaluation, calculated as the mean of all quality control (n = 3) values obtained throughout the entire five-day protocol.

Table 4 summarises all data on precision and accuracy.

3.2.4. *Recovery*

To evaluate the recovery capability of the method, a protocol was developed using three concentrations—low, medium, and high—for each analyte (0.2, 1 and 5 ng/mg for KET; 0.8, 3 and 5 ng/mg for NKET). By fortifying the sample with a mixture of analytes before and after the clean-up procedure, this parameter could be analysed. The recovery rates ranged from 47–76 % for KET and 14–27 % for NKET, which were considered satisfactory (Table 5).

The developed method demonstrated high sensitivity with a low sample volume under miniaturized conditions, a crucial consideration

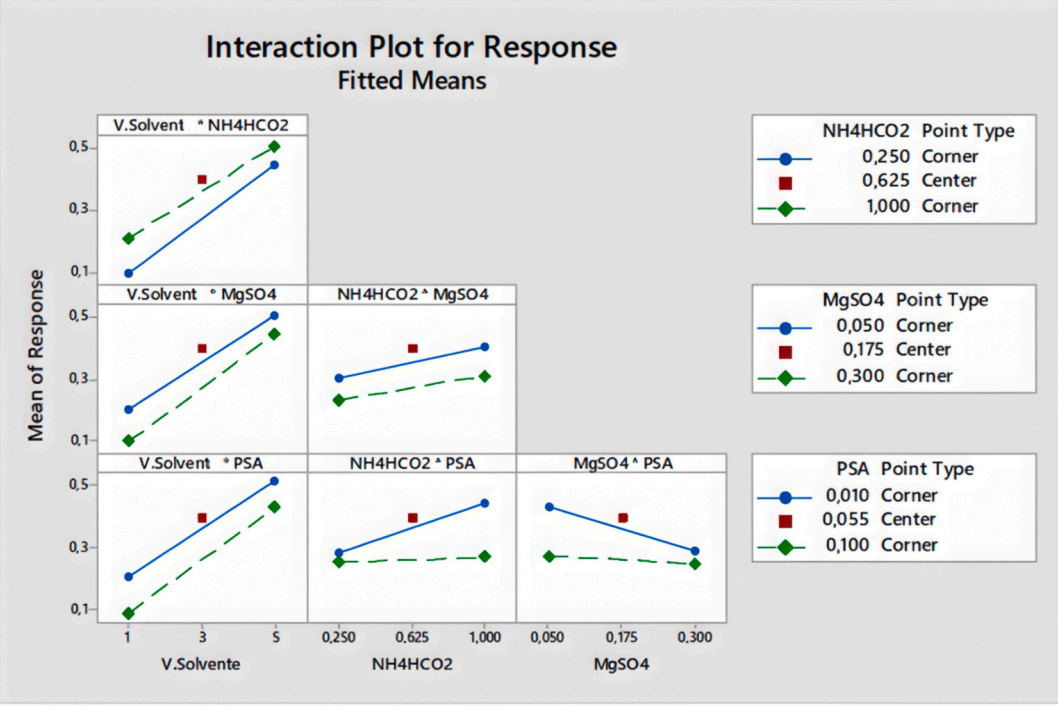

Fig. 3. Interaction plot for response.

Table 3
Linearity data (n = 5).

Analyte	Regression model	Working range (ng/mg)	Slope	Intercept	R2	LLOQ (ng/mg)	LOD (ng/mg)
KET	1/x	0.05–5	5.1609± 1.5288	−0.1532± 0.1341	0.9938± 0.0034	0.05	0.01
NKET		0.8–5	0.1833± 0.0337	−0.0117± 0.0086	0.9943± 0.0025	0.8	0.8

when designing environmentally sustainable methods. To provide a clearer perspective on its performance, a comparison with other studies is presented in Table 6. The comparison focused on methods targeting the same analytes, utilizing the same matrix, and employing MS/MS for the detection of KET and NKET.

The first method presented [1], focused on the extraction of KET and other analytes from 10 mg of hair using a methanolic extraction, assisted by microwave and trifluoroacetic acid (TFA). Although the LLOQ, LOD and recoveries from a small sample amount are interesting, it is impossible to overlook the use of a toxic reagent like TFA. Zhuo et al. [2], also achieved noticeable results, however had to increase the complexity of the extraction methodology with cryogenic grinding, making the method less captivating in the routine perspective. Other studies [3,4], focused on the use of SPE for the extraction of this NPS and its metabolite, using a smaller amount of hair than the presented in this study, whilst achieving lower values for the LOD and LLOQ. However, one of the major disadvantages of SPE cartridges is the cost, and these materials are not reusable creating a large footprint per extraction. Besides, all the above methods and others that achieve fascinating results via liquid base extractions with small volumes of solvents and sample [5–7] use LC-MS/MS systems as the chromatographic solution. Although, as shown, this equipment allows obtaining great results due to associated technologic advancements, it is impossible to overlook the cost of these units, not only for acquiring but also for maintenance. Besides this, LC uses great amounts of solvents for the mobile phase and thus creates a large amount of waste. Additionally, a microextraction by packed sorbent (MEPS)-based approach provides a solution with high sensitivity that uses low amount of solvents and by reutilization, is a low-cost clean-up technique that creates small amounts of waste, and by associating with GC–MS/MS, the analysis cost is also much more interesting when compared to LC. However, its adaptation to routine analysis presents challenges, as manual MEPS allows processing only one sample at a time, which leads to a rapidly increasing workload when handling multiple samples [8].

These characteristics demonstrate the hurdle of combining green, safe, applicability in routine and cost-effective techniques in a single methodology.

The μ-QuEChERS method, in contrast, supports the processing of multiple samples simultaneously without compromising workflow efficiency. It generates minimal waste and avoids the use of highly toxic reagents, thanks to the miniaturization of the QuEChERS technique. Moreover, the sample pre-treatment stage does not require complex equipment, making it accessible and user-friendly, and importantly, this developed method achieved low LLOQ and LOD values, an appropriate working range, and retained a green and comparatively cheap methodology.

Beyond its environmental and cost benefits, the method is versatile and can be easily applied to a wide range of sample types. This indicates that it is a feasible miniaturized solution that could be seamlessly integrated into routine forensic workflows. Furthermore, the method's linear range incorporates the cut-off value for KET (0.2 ng/mg) as proposed by the SOHT.

3.3. Method application to real samples

The developed method was applied to hair samples from individuals

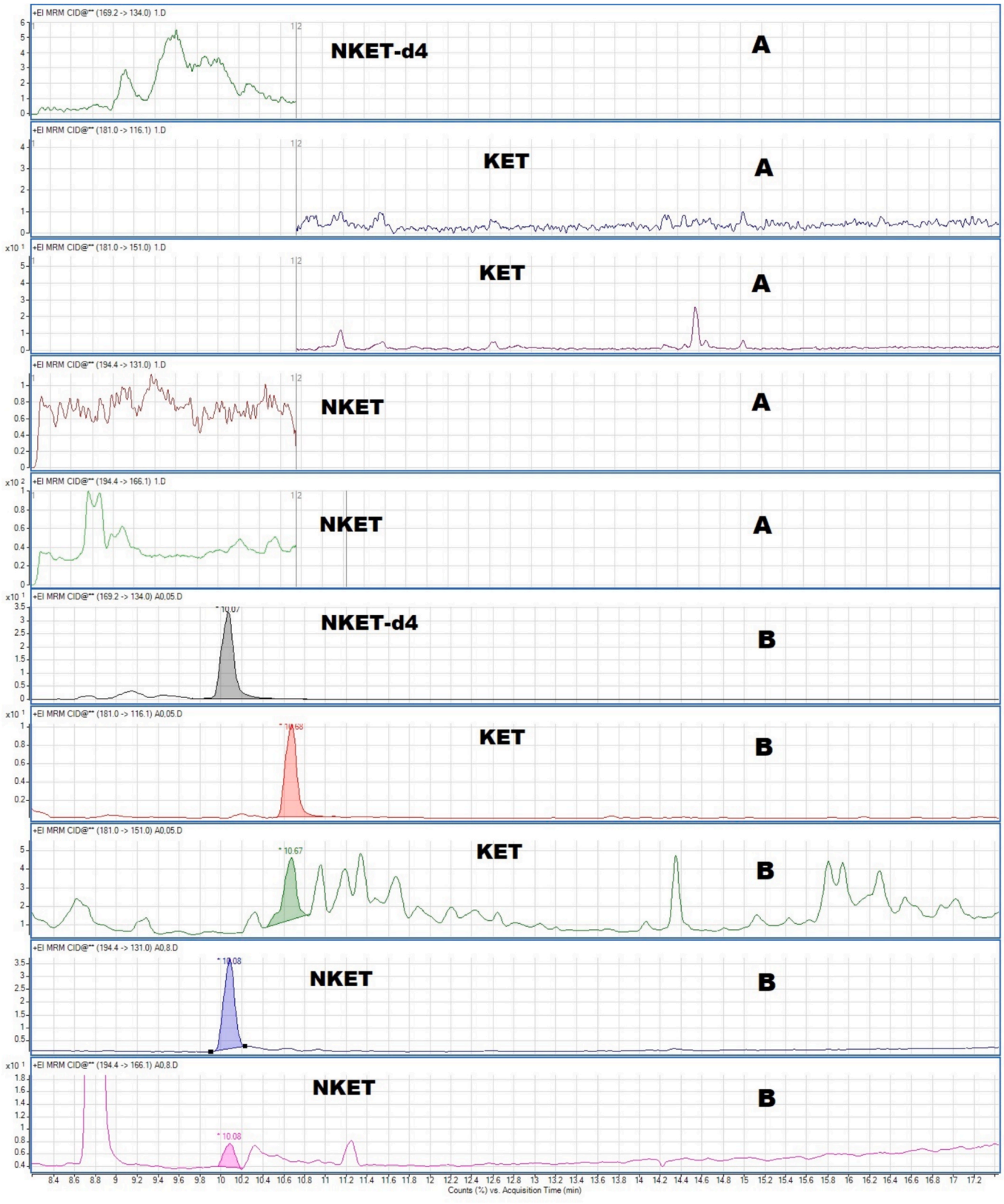

Fig. 4. Chromatograms of selected fragments obtained after extraction of a blank: (A) and a spiked at the LLOQ (B).

who attended an alternative music festival in Portugal in 2023. Table 7 describes the concentrations found for both analytes.

These values provide a significant basis for interpreting drug consumption. For instance, in the first sample, the high concentration of the main drug allows for the inference of possible chronic use, given that the values reported in the literature for consumers range between 0.1 and 99.3 ng/mg [19]. In the remaining samples, KET was detected, but it was below the cut-off values proposed by the SOHT. NKET was not detected

Table 4
Precision and accuracy.

Parameter	Analyte	Nominal concentration (ng/mg)	CV%	BIAS%
Intraday (n = 5)	KET	0.05	9.28	7.16
		0.2	17.51	−6.12
		1	12.70	−0.47
		5	14.47	5.38
	NKET	0.8	9.35	−2.23
		1	4.95	−0.22
		3	7.44	11.67
		5	6.21	6.59
Intermediate (n = 15)	KET	0.05	5.18	3.01
		0.2	12.46	−5.98
		1	12.12	−10.83
		5	14.20	−13.47
	NKET	0.8	3.79	−2.83
		1	1.94	−0.11
		3	10.50	−7.36
		5	2.28	1.71
Interday (n = 5)	KET	0.05	7.59	5.16
		0.2	14.93	−10.43
		1	13.10	−8.83
		5	1.07	0.77
	NKET	0.8	8.59	6.37
		1	9.61	−7.01
		3	3.58	2.60
		5	3.40	2.50

Table 5
Recoveries (n = 3).

Analyte	Concentration (ng/mg)	Recovery %
KET	0.2	76.35 ± 3.16
	1	52.43 ± 3.45
	5	47.47 ± 4.56
NKET	0.8	14.43 ± 4.25
	3	20.82 ± 2.34
	5	27.05 ± 1.61

in any sample. To verify the performance of the proposed method, these same samples were sent to an accredited laboratory for hair analysis. The concentrations obtained from the real samples were identical to those obtained using the proposed method.

4. Conclusion

This study focused on developing a novel clean-up technique for hair samples by adapting an approach previously utilized in the analytical fields. Due to the complexity of hair as a matrix, the QuEChERS method proved highly effective in concentrating KET, achieving exceptional sensitivity when properly executed.

Through careful optimization of the pretreatment process, the miniaturization of the clean-up method was successfully accomplished, surpassing initial expectations for the technique. Achieving a miniaturized method that is both user-friendly and capable of delivering high sensitivity is a rare achievement, as most miniaturized techniques face challenges in maintaining adequate sensitivity when applied to routine analysis. This progress represents a notable contribution to green chemistry.

The method validation demonstrated its robustness, with detection and quantification limits, and linearity comparable to existing processes. Its successful application to real samples further confirmed the method's effectiveness.

These findings are promising, and this approach may be applied to the analysis of other drugs of abuse in hair samples. The key factor appears to be the proper optimisation of the QuEChERS protocol, which has helped overcoming analysis hurdles, namely those related to the high level of complexity of biological samples.

To our knowledge, this is the first study to utilize μ-QuEChERS as a clean-up method for detecting KET and NKET in hair, opening a new path for future research in this field.

Table 7
KET and NKET concentrations found in real hair samples.

Sample	Concentration (ng/mg)		Accredited Laboratory concentration (ng/mg)	
	KET	NKET	KET	NKET
1	12.55	Not detected	15.89	Not detected
2	Not detected	Not detected	Not detected	Not detected
3	< LLOQ	Not detected	< LLOQ (0.05)	Not detected
4	0.15	Not detected	0.11	Not detected
5	0.12	Not detected	0.10	Not detected
6	0.05	Not detected	0.06	Not detected

Table 6
Methods to determine KET and NKET in hair samples.

Analytes	Hair amount (mg)	Extraction technique	Clean-up technique	Linear range (ng/mg)	LLOQ (ng/mg)	LOD (ng/mg)	Recovery	Chromatographic technique	Reference
KET NKET	10	Microwave-assisted extraction with methanol and TFA (85:15)	N.S.	0.002–1	0.002	0.0005	91–98 94–98	LC-MS/MS	[21]
KET NKET	10	Methanolic extraction	N.S.	0.05–5	0.05	0.02	75–94 92–95	UHPLC-MS/MS	[42]
KET NKET	50	Methanolic extraction with cryogenic grinding	N.S.	0.05–10	0.05	0.02	92–108 72–93	UHPLC-MS/MS	[22]
KET NKET	30	Extraction with phosphate buffer (pH 6)	Online SPE (Strata-X-C Mixed-mode)	0.02–10 0.04–4	0.02 0.04	0.008 0.015	103–104 96–100	LC-MS/MS	[24]
KET NKET	3	Bond elut SPE	N.S.	0.01–10	0.01	0.001	N.S.	LC-MS/MS	[23]
KET NKET	2.5	Extraction with methanol: acetonitrile: 20 mM ammonium formate (25:25:50)	N.S.	0.1–200	0.1	0.02	97 ± 15 96 ± 25	LC-MS/MS	[19]
KET NKET	20	Extraction with 0.01 % formic acid	N.S.	0.5–25	0.5	0.1	66–87 85–99	LC-MS/MS	[20]
KET NKET	50	Methanolic extraction	MEPS M1 (4 mg; 80 % C8 and 20 % SCX)	0.05–10	0.05	0.01 0.05	39–60 32–43	GC–MS/MS	[43]
KET NKET	50	Methanolic extraction	μ-QuEChERS	0.05–5 0.8–5	0.05 0.8	0.01 0.8	47–76 14–27	GC–MS/MS	This work

5. Informed Consent Statement.

This study was approved by the Ethics Committees of the Centro Hospitalar Universitário Cova da Beira and the University of Beira Interior, and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all subjects involved in the study.

CRediT authorship contribution statement

Rodrigo Pelixo: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Mário Barroso:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Tiago Rosado:** Writing – review & editing, Validation, Supervision, Formal analysis, Conceptualization. **Eugenia Gallardo:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was partially supported by CICS-UBI, which is financed by National Funds from Fundação para a Ciência e a Tecnologia (FCT) and by Fundo Europeu de Desenvolvimento Regional (FEDER) under the scope of PORTUGAL 2020 and Programa Operacional do Centro (CENTRO 2020), with the project references https://doi.org/10.54499/UIDB/00709/2020 and https://doi.org/10.54499/UIDP/00709/2020.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2025.113770.

Data availability

Data will be made available on request.

References

[1] United States Drug Enforcement Administration Diversion Control Division, PHENCYCLIDINE (Street Names: PCP, Angel Dust, Supergrass, Boat, Tic Tac, Zoom, Shermans), 2023.
[2] R.E. Garey, L.A. Weisberg, R.G. Heath, Phencyclidine: An overview, J Psychoactive Drugs 9 (1977) 280–285, https://doi.org/10.1080/02791072.1977.10472059.
[3] R.C. Petersen, R.C. Stillman, Phencyclidine (PCP) Abuse: An Appraisal Editors, in: National Institute on Drug Abuse Research Monograph 21, Department of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, National Institute on Drug Abuse, Division of Research, 1978.
[4] G. Corssen, M.D. Edward, A. Arbor, F. Domino, Dissociative Anesthesia: Further Pharmacologic Studies and First Clinical Experience with Phencyclidine Derivate CI-581, Anesthesia and Analgesia Current Researches (1966). http://journals.lww.com/anesthesia-analgesia.
[5] L. Li, P.E. Vlisides, Ketamine: 50 years of modulating the mind, Front Hum Neurosci 10 (2016), https://doi.org/10.3389/fnhum.2016.00612.
[6] T.V. Chaves, B. Wilffert, Z.M. Sanchez, Overdoses and deaths related to the use of ketamine and its analogues: a systematic review, Am. J. Drug Alcohol Abuse 49 (2023) 141–150, https://doi.org/10.1080/00952990.2022.2132506.
[7] D. Baumeister, L.M. Tojo, D.K. Tracy, Legal highs: Staying on top of the flood of novel psychoactive substances, Ther Adv Psychopharmacol 5 (2015) 97–132, https://doi.org/10.1177/2045125314559539.
[8] R. Pelletier, B. Le Daré, D. Le Bouëdec, A. Kernalléguen, P.J. Ferron, I. Morel, T. Gicquel, Arylcyclohexylamine Derivatives: Pharmacokinetic, Pharmacodynamic, Clinical and Forensic Aspects, Int J Mol Sci 23 (2022), https://doi.org/10.3390/ijms232415574.
[9] United States Drug Enforcement Administration Diversion Control Division, Ketamine, 2024.
[10] European Union Drug Agency, EUROPOL, EU Drug Market: New psychoactive substances-Distribution and supply in Europe: Ketamine Background, n.d. https://www.euda.europa.eu/publications/eu-drug-markets/new-psychoactive-substances/distribution-and-supply/ketamine_en (accessed October 9, 2024).
[11] P. Zanos, R. Moaddel, P.J. Morris, L.M. Riggs, J.N. Highland, P. Georgiou, E.F. R. Pereira, E.X. Albuquerque, C.J. Thomas, C.A. Zarate, T.D. Gould, Ketamine and ketamine metabolite pharmacology: Insights into therapeutic mechanisms, Pharmacol Rev 70 (2018) 621–660, https://doi.org/10.1124/pr.117.015198.
[12] K. Wolff, A.R. Winstock, Ketamine From Medicine to Misuse, CNS Drugs 20 (2006) 199–218, https://doi.org/10.2165/00023210-200620030-00003.
[13] T. Andrew Bowdle, A.D. Radant, D.S. Cowley, E.D. Kharasch, R.J. Strassman, P. P. Roy-Byrne, Psychedelic Effects of Ketamine in Healthy Volunteers, Anesthesiology (1998), https://doi.org/10.1097/00000542-199801000-00015.
[14] Y.R.de.S. Costa, S.N. Lavorato, J.J.C.M.de C. Baldin, Violence against women and drug-facilitated sexual assault (DFSA): A review of the main drugs, J Forensic Leg Med 74 (2020), https://doi.org/10.1016/j.jflm.2020.102020.
[15] M. Barroso, E. Gallardo, Hair analysis for forensic applications: Is the future bright? Bioanalysis 6 (2014) 1–3, https://doi.org/10.4155/bio.13.291.
[16] E. Gallardo, J.A. Queiroz, The role of alternative specimens in toxicological analysis, Biomed. Chromatogr 22 (2008) 795–821, https://doi.org/10.1002/bmc.1009.
[17] P. Xiang, M. Shen, O.H. Drummer, Review: Drug concentrations in hair and their relevance in drug facilitated crimes, J Forensic Leg Med 36 (2015) 126–135, https://doi.org/10.1016/j.jflm.2015.09.009.
[18] D. Favretto, G. Cooper, M. Andraus, F. Sporkert, R. Agius, B. Appenzeller, M. Baumgartner, T. Binz, V. Cirimele, R. Kronstrand, M. del Mar Ramirez, S. Strano-Rossi, M. Uhl, M. Vincenti, M. Yegles, The Society of Hair Testing consensus on general recommendations for hair testing and drugs of abuse testing in hair, Drug Test Anal 15 (2023) 1042–1046, https://doi.org/10.1002/dta.3526.
[19] K.W. Leung, Z.C.F. Wong, J.Y.M. Ho, A.W.S. Yip, J.S.C. Ng, S.P.H. Ip, W.Y.Y. Ng, K. K.L. Ho, R. Duan, K.Y. Zhu, K.W.K. Tsim, Determination of hair ketamine cut-off value from Hong Kong ketamine users by LC-MS/MS analysis, Forensic Sci Int 259 (2016) 53–58, https://doi.org/10.1016/j.forsciint.2015.12.004.
[20] M.J. Tabernero, M.L. Felli, A.M. Bermejo, M. Chiarotti, Determination of ketamine and amphetamines in hair by LC/MS/MS, Anal Bioanal Chem 395 (2009) 2547–2557, https://doi.org/10.1007/s00216-009-3163-4.
[21] Y.J. Chang, M.R. Chao, S.C. Chen, C.H. Chen, Y.Z. Chang, A high-throughput method based on microwave-assisted extraction and liquid chromatography-tandem mass spectrometry for simultaneous analysis of amphetamines, ketamine, opiates, and their metabolites in hair, Anal Bioanal Chem 406 (2014) 2445–2455, https://doi.org/10.1007/s00216-014-7669-z.
[22] Y. Zhuo, X. Wang, J. Wu, S. Zhang, H. Deng, W. Liu, H. Wu, P. Xiang, M. Shen, Simultaneous Quantitative Determination of Amphetamines, Opiates, Ketamine, Cocaine and Metabolites in Human Hair: Application to Forensic Cases of Drug Abuse, J Forensic Sci 65 (2020) 563–569, https://doi.org/10.1111/1556-4029.14179.
[23] J. Rhee, J. Lee, J. Kim, M. Jang, I. Shin, S. Lee, Determination of Ketamine and Norketamine in Hair and Evaluation of Polydrug Use in Ketamine Abusers Using Hair Analysis in Korea, J Anal Toxicol 45 (2021) 993–1005, https://doi.org/10.1093/jat/bkaa166.
[24] A.S. Barreto, V.F. Brant, E. Spinelli, S.V. Rodrigues, Validation of a SPE-LC–MS/MS method for the determination of ketamine and norketamine in micropulverized hair after a single IV dose, J Chromatogr B Analyt Technol Biomed Life Sci 1033–1034 (2016) 200–209, https://doi.org/10.1016/j.jchromb.2016.08.027.
[25] M. Anastassiades, S.J. Lehotay, Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce, J AOAC Int 86 (2003), https://doi.org/10.1093/jaoac/86.2.412.
[26] R. Perestrelo, P. Silva, P. Porto-Figueira, J.A.M. Pereira, C. Silva, S. Medina, J. S. Câmara, QuEChERS - Fundamentals, relevant improvements, applications and future trends, Anal Chim Acta 1070 (2019) 1–28, https://doi.org/10.1016/j.aca.2019.02.036.
[27] S.J. Lehotay, M. O', N.; Josée, T.; Antonio, V. García, M. Contreras, H. Mol, V. Heinke, T. Anspach, G. Lach, R. Fussell, K. Mastovska, ; Mette, E. Poulsen, A. Brown, W. Hammack, J.M. Cook, ; Lutz, K. Lindtner, M. Gamón Vila, M. Hopper, A. De Kok, M. Hiemstra, F. Schenck, A. Williams, A. Parker, Determination of Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate: Collaborative Study, LEHOTAY: JOURNAL OF AOAC INTERNATIONAL 90 (2007) 485. http://www.atypon-link.com/AOAC/loi/jaoi.
[28] A. Spietelun, Ł. Marcinkowski, M. de la Guardia, J. Namieśnik, Recent developments and future trends in solid phase microextraction techniques towards green analytical chemistry, J Chromatogr A 1321 (2013) 1–13, https://doi.org/10.1016/j.chroma.2013.10.030.
[29] B. Yuan, D. Zhao, W. Lyu, Z. Yin, D. Kshatriya, J.E. Simon, N.T. Bello, Q. Wu, Development and validation of a micro-QuEChERS method with high-throughput enhanced matrix removal followed with UHPLC-QqQ-MS/MS for analysis of raspberry ketone-related phenolic compounds in adipose tissues, Talanta 235 (2021), https://doi.org/10.1016/j.talanta.2021.122716.
[30] M. Kusano, Y. Sakamoto, Y. Natori, H. Miyagawa, H. Tsuchihashi, A. Ishii, K. Zaitsu, Development of "Quick-DB forensic": A total workflow from QuEChERS-dSPE method to GC–MS/MS quantification of forensically relevant drugs and pesticides in whole blood, Forensic Sci Int 300 (2019) 125–135, https://doi.org/10.1016/j.forsciint.2019.03.048.
[31] S. Dulaurent, S. El Balkhi, L. Poncelet, J.M. Gaulier, P. Marquet, F. Saint-Marcoux, QuEChERS sample preparation prior to LC-MS/MS determination of opiates, amphetamines, and cocaine metabolites in whole blood, Anal Bioanal Chem 408 (2016) 1467–1474, https://doi.org/10.1007/s00216-015-9248-3.

[32] J.L. Westland, F.L. Dorman, QuEChERS extraction of benzodiazepines in biological matrices, J Pharm Anal 3 (2013) 509–517, https://doi.org/10.1016/j.jpha.2013.04.004.
[33] S. Matsuta, K. Nakanishi, A. Miki, K. Zaitsu, N. Shima, T. Kamata, H. Nishioka, M. Katagi, M. Tatsuno, K. Tsuboi, H. Tsuchihashi, K. Suzuki, Development of a simple one-pot extraction method for various drugs and metabolites of forensic interest in blood by modifying the QuEChERS method, Forensic Sci Int 232 (2013) 40–45, https://doi.org/10.1016/j.forsciint.2013.06.015.
[34] J. Kim, H.D. Cho, J.H. Suh, J.Y. Lee, E. Lee, C.H. Jin, Y. Wang, S. Cha, H. Im, S. B. Han, Analysis of nicotine metabolites in hair and nails using QuEChERS method followed by liquid chromatography-tandem mass spectrometry, Molecules 25 (2020), https://doi.org/10.3390/molecules25081763.
[35] M. Licata, C. Rustichelli, F. Palazzoli, A. Ferrari, C. Baraldi, D. Vandelli, P. Verri, F. Marchesi, E. Silingardi, Hair testing in clinical setting: Simultaneous determination of 50 psychoactive drugs and metabolites in headache patients by LC tandem MS, J Pharm Biomed Anal 126 (2016) 14–25, https://doi.org/10.1016/j.jpba.2016.04.015.
[36] E. Lehmann, C. Oltramare, L.F. de Alencastro, Development of a modified QuEChERS method for multi-class pesticide analysis in human hair by GC-MS and UPLC-MS/MS, Anal Chim Acta 999 (2018) 87–98, https://doi.org/10.1016/j.aca.2017.11.009.
[37] P.K. Grover, R.L. Ryall, Critical appraisal of salting-out and its implications for chemical and biological sciences, Chem Rev 105 (2005) 1–10, https://doi.org/10.1021/cr030454p.
[38] G. Islas, I.S. Ibarra, P. Hernandez, J.M. Miranda, A. Cepeda, Dispersive Solid Phase Extraction for the Analysis of Veterinary Drugs Applied to Food Samples: A Review, Int J Anal Chem 2017 (2017), https://doi.org/10.1155/2017/8215271.
[39] J. Gonçalves, T. Rosado, M. Barroso, J. Restolho, N. Fernández, Á. Luís, E. Gallardo, A.P. Duarte, Comparative study of sample preparation procedures to determine the main compounds in ayahuasca beverages by QuEChERS and high-performance liquid chromatography analysis, Phytochem. Anal (2024), https://doi.org/10.1002/pca.3370.
[40] Z. Zhang, H. Cheng, Recent Development in Sample Preparation and Analytical Techniques for Determination of Quinolone Residues in Food Products, Crit Rev Anal Chem 47 (2017) 223–250, https://doi.org/10.1080/10408347.2016.1266924.
[41] Standard Practices for Method Validation in Forensic Toxicology, Colorado Springs, 2019. www.asbstandardsboard.org.
[42] S. Chen, J. Ma, X. Wang, P. Geng, UPLC-MS/MS Simultaneous Determination of Methamphetamine, Amphetamine, Morphine, Monoacetylmorphine, Ketamine, Norketamine, MDMA, and MDA in Hair, Acta Chromatogr 32 (2020) 145–148, https://doi.org/10.1556/1326.2019.00615.
[43] A.Y. Simão, P. Oliveira, L.M. Rosendo, T. Rosado, M. Andraus, M. Barroso, E. Gallardo, Microextraction by Packed Sorbent as a Clean-up Approach for the Determination of Ketamine and Norketamine in Hair by Gas Chromatography–Tandem Mass Spectrometry, J Anal Toxicol 47 (2023) 227–235, https://doi.org/10.1093/jat/bkac075.