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Avaliação da microextração líquido-líquido homogênea com solventes de hidrofilicidade comutável na determinação de MDMA, MDA e NBOMes em amostras de sangue *postmortem*

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RESUMO

As drogas sintéticas para fins recreativos estão em constante evolução e seu consumo representa um grande risco à saúde pública. O desenvolvimento de metodologias analíticas para comprovar o consumo de drogas ilícitas em fluidos biológicos torna-se necessário para o controle dessas substâncias. Neste estudo, foi proposto, pela primeira vez, um método de extração envolvendo microextração líquido-líquido homogênea utilizando solvente de hidrofilicidade comutável como fase extratora (SHS-HLLME) para a determinação das drogas sintéticas MDMA, MDA e NBOMes (25B, 25C e 25I) em sangue total e posterior análise por cromatografia líquida acoplada a espectrometria de massas sequencial (LC-MS / MS). A otimização das principais variáveis do preparo de amostras foi realizada de forma multivariada. As condições ótimas de extração consistiram no uso de 250 µL de ZnSO4 10% e 50 µL de NaOH 1 mol L^{-1} na etapa de precipitação de proteínas; N,N-dimetilciclohexilamina (DMCHA) como solvente de hidrofilicidade comutável, 650 µL da mistura de SHS:HCl 6 mol L^{-1} (1:1 v/v), 500 µL de amostra de sangue total, 500 µL de NaOH 10 mol L^{-1} e 1 min de tempo de extração. O método proposto foi validado, fornecendo coeficientes de determinação superiores a 0.99 para todos os analitos; Limites de detecção (LOD) e limites de quantificação (LOQ) de 0,1 a 10 ng mL^{-1}; precisão intra-corrida de 2,16 a 9,19%; precisão inter-corrida de 2,39 a 9,59%; exatidão de 93.57 a 115.71%; e supressão da ionização de 28,94 a 51,54%. Esta metodologia foi aplicada em quatro amostras de sangue *post-mortem* provenientes do Departamento de Perícias Laboratoriais do Instituto Geral de Perícias (DPL-IGP) do Rio Grande do Sul e a presença de MDA e MDMA foi identificada em concentrações de 31,76 a 61,60 ng mL^{-1} e 6,91 a 68,20 ng mL^{-1}, respectivamente. O método desenvolvido é uma alternativa ecologicamente correta para a análise toxicológica dessas drogas sintéticas em amostras de sangue total humano obtidas de indivíduos com suspeita de intoxicação e essa técnica apresenta grande potencial para ser aplicada em determinações forenses.

***Palavras-chave*:** NBOMes; estimulantes tipo anfetamina; novas substâncias psicoativas (NSP); microextração líquido-líquido homogênea com solvente de hidrofilicidade comutável (SHS-HLLME); sangue total; cromatografia líquida acoplada à espectrometria de massas sequencial (LC-MS/MS).

ABSTRACT

Synthetic drugs for recreational purposes are in constant evolution and their consumption constitutes a significant risk to public health. The development of an analytical methodology to confirm the intake of illicit drugs in biological fluids is required for control of these substances. In this study, it was proposed, for the first time, an extraction method based on homogenous liquid-liquid microextraction with switchable hydrophilicity solvent as extraction phase (SHS-HLLME) for the determination of the synthetic drugs MDMA, MDA and NBOMes (25B, 25C and 25I) in whole blood, followed by liquid chromatography coupled to mass spectrometry in tandem (LC-MS/MS). The optimization of the main sample preparation variables was performed through multivariate approaches. The optimized sample preparation conditions consisted of using 250 μL of $ZnSO_4$ 10% and 50 μL of NaOH 1 mol L^{-1} in the protein precipitation step; N,N-dimethylcyclohexylamine (DMCHA) was used as switchable hydrophilicity solvent (SHS), 650 μL of a mixture of SHS:HCl 6 mol L^{-1} (1:1 v/v), 500 μL of whole blood sample, 500 μL of NaOH 10 mol L^{-1} and 1 min of extraction time. The proposed method was validated, providing determination coefficients higher than 0.99 for all analytes; limit of detection (LOD) and limit of quantification (LOQ) ranged from 0.1 to 10 ng mL^{-1}; intra-run precision from 2.16 to 9.19%; inter-run precision from 2.39 to 9.59%; bias from 93.57% to 115.71%; and matrix effect suppression from 28.94 to 51.54%. The developed method was applied in four post-mortem blood samples from Departamento de Perícias Laboratoriais do Instituto Geral de Perícias (DPL-IGP), Rio Grande do Sul, and the presence of MDA and MDMA was identified in concentrations from 31.76 to 61.60 ng mL^{-1} and 6.91 to 68.20 ng mL^{-1}, respectively. The developed method is an environmentally friendly alternative for toxycological analyses of these synthetic drugs in human whole blood samples obtained from individuals suspected of intoxication and this technique exhibits great potential to be applied in forensic determinations.

***Keywords*:** NBOMes; amphetamine-type stimulants; new psychoactive substances (NPS); Switchable Hydrophilicity Solvent Homogeneous Liquid-Liquid Microextraction (SHS-HLLME); whole blood; liquid chromatography tandem mass spectrometry (LC-MS/MS).

LISTA DE ABREVIATURAS E SIGLAS

µP-SHS-HLLME Microextração Líquido-Líquido Homogênea com Solvente de Hidrofilicidade Comutável em µ-Pipeta, do inglês: *µ-Pipette - Switchable Hydrophilicity Solvent - Homogeneous Liquid-Liquid Microextraction*

ANVISA Agência Nacional de Vigilância Sanitária

ATS Estimulantes tipo anfetamina, do inglês: *amphetamine-type stimulants*

DBU 1,8-Diazabicyclo [5.4.0] undec-7-eno

DLLME Microextração Líquido-Líquido Dispersiva, do inglês: *Dispersive Liquid-Liquid Microextraction*

DMBA N,N-dimetilbenzilamina

DMCHA N,N-dimetilciclohexilamina

DPA Dipropilamina

DPV Voltametria de Pulso Diferencial, do inglês: *Differential pulse voltammetry*

EA-SS-LPME Microextração em Fase Líquida baseada em Solvente Comutável Assistida por Efervescência, do inglês: *Effervescence-Assisted Switchable Solvent-Based Liquid Phase Microextraction.*

EMCDDA Centro de Monitoramento Europeu para Drogas e Toxicodependência, do inglês: *European Monitoring Centre for Drugs Addiction*

EHPA Ácido di(2-etil-hexil) fosfórico

ETAAS Espectroscopia de Absorção Atômica Eletrotérmica, do inglês: *Electrothermal Atomic Absorption Spectroscopy*

FAAS Espectrometria de Absorção Atômica com chama, do inglês: *Flame-atomic absorption spectrometry*

GC-FID Cromatografia Gasosa com Detector por Ionização de Chama, do inglês: *Gas Chromatography-Flame Ionization Detector*

GC-MS	Cromatografia Gasosa acoplada à Espectrometria de Massas, do inglês: *Gas Chromatography – Mass Spectrometry*
GC-MS/MS	Cromatografia Gasosa acoplada à Espectrometria de Massas em tandem, do inglês: *Gas Chromatography – Tandem Mass Spectrometry*
GFAAS	Espectrometria de Absorção Atômica com forno de grafite, do inglês: *Graphite Furnace Atomic Absorption Spectroscopy*
HexA	Ácido Hexanóico
HLLME	Microextração Líquido-Líquido Homogênea, do inglês: *Homogeneous Liquid-Liquid Microextraction*
HPLC-DAD	Cromatografia Líquida de Alta Eficiência acoplada ao Detector de Arranjo de Diodos, do inglês: *High Performance Liquid Chromatography – Diode Array Detector*
HPLC-FLD	Cromatografia Líquida de Alta Eficiência acoplada ao Detector de Fluorescência, do inglês: *High Performance Liquid Chromatography – Fluorescence detector*
HPLC–UV	Cromatografia Líquida de Alta Eficiência acoplada ao Detector de Ultravioleta, do inglês: *High Performance Liquid Chromatography – Ultraviolet detector*
IP-SHS-HLLME	Microextração Líquido-Líquido Homogênea com Solvente de Hidrofilicidade Comutável e Par Iônico, do inglês: *Ion-Pair Switchable Hydrophilicity Solvent-Based Homogeneous Liquid-Liquid Microextraction*
LC-MS/MS	Cromatografia Líquida acoplada à Espectrometria de Massas Sequencial, do inglês: *Liquid Chromatography – Tandem Mass Spectrometry*
LLE	Extração Líquido-Líquido, do inglês: *Liquid-Liquid Extraction*
LOD	Limite de Detecção, do inglês: *Limit of Detection*
LOQ	Limite de Quantificação, do inglês: *Limit of Quantitation*
LSD	Dietilamida do ácido lisérgico, do inglês: *Lysergic Acid Diethylamide*
MDA	Tenamfetamina, do inglês: *3,4-Methylenedioxyamphetamine*
MDMA	Metilenodioximetanfetamina, do inglês: *3,4-Methylenedioxymethamphetamine*

NSP	Novas Substâncias Psicoativas
OA	Ácido Octanóico
PF	Polícia Federal
SIP-SS-DLLME	Microextração Líquido-Líquido Homogênea com Solvente de Hidrofilicidade Comutável e Par Iônico Surfactante, do inglês: *Surfactant ion Pair Switchable Solvent Dispersive Liquid–Liquid Microextraction*
SHS	Solvente com Hidrofilicidade Comutável, do inglês: *Switchable Hydrophilicity Solvent*
SHS-BME	Microextração baseada em Solvente com Hidrofilicidade Comutável, do inglês: *Switchable Hydrophilicity Solvent-based Microextraction*
SHS-HLLME	Microextração Líquido-Líquido Homogênea com Solvente de Hidrofilicidade Comutável, do inglês: *Switchable Hydrophiliciy Solvent Homogeneous Liquid-Liquid Microextraction*
SHS-LLME	Microextração Líquido-Líquido com Solvente de Hidrofilicidade Comutável, do inglês: *Switchable Hydrophilicity Solvent - Liquid-Liquid Microextraction*
SHS-LPME	Microextração em Fase Líquida com Solvente de Hidrofilicidade Comutável, do inglês: *Switchable Hydrophilicity Solvent – Liquid Phase Microextraction*
SM-LPME	Microextração em Fase Líquida com Membrana por Agitação, do inglês: *Stir Membrane – Liquid Phase Microextraction*
SS-HLLME	Microextração Líquido-Líquido Homogênea com Solvente Comutável, do inglês: *Switchable Solvent - Homogeneous Liquid Liquid Microextraction*
SS-LPME	Microextração em Fase Líquida com Solvente Comutável, do inglês: *Switchable Solvent – Liquid Phase Microextraction*
SS-LPME-SFO	Microextração em Fase Líquida com Solvente Comutável com Solidificação de Gotas Orgânicas Flutuantes, do inglês: *Switchable solvent liquid-phase microextraction technique based on the solidification of floating organic droplets*
SPE	Extração em Fase Sólida, do inglês: *Solid Phase Extraction*
SWGTOX	Grupo de Trabalho Científico de Toxicologia Forense, do inglês: *Scientific Working Group for Forensic Toxicology*

TEA	Trietilamina
THC	Tetrahidrocanabinol
UHPLC-ESI-MS/MS	Cromatografia líquida de ultra eficiência acoplada à espectrometria de massas sequencial e ionização por electrospray, do inglês: *Ultra-high performance liquid Chromatography - electrospray ionization - tandem mass spectrometry*
UNODC	Escritório das Nações Unidas sobre Drogas e Crimes, do inglês: *United Nations Office on Drugs and Crime*
UV/VIS	Espectrofotometria no Ultravioleta-visível

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1. INTRODUÇÃO

1.1 DROGAS SINTÉTICAS: ESTIMULANTES TIPO ANFETAMINA E NOVAS SUBSTÂNCIAS PSICOATIVAS

O uso de substâncias psicoativas é considerado um importante problema de saúde pública global, que implica em diversas consequências sociais e de saúde para a população (MARTINOTTI et al., 2020). Conforme o Relatório Mundial sobre Drogas de 2020, cerca de 269 milhões de pessoas no mundo, na faixa entre 15 e 64 anos, usaram drogas pelo menos uma vez no ano de 2018. Deste total de usuários, estima-se que 35,6 milhões sofram de transtornos relacionados ao uso de drogas, evidenciando que a utilização destas substâncias é prejudicial a ponto de causar dependência e/ou requerer tratamento (UNODC, 2020).

No Brasil, a Agência Nacional de Vigilância Sanitária (ANVISA) é o órgão governamental responsável pela classificação de drogas lícitas e ilícitas. Na portaria Nº 344, de 12 de maio de 1998, é possível encontrar as substâncias e medicamentos de controle especial e proscritos no país. As substâncias de interesse forense estão dispostas na Lista F (substâncias de uso proscrito no Brasil), que apresenta os subgrupos F1 (entorpecentes, como cocaína), F2 (psicotrópicas, como tetrahidrocanabinol – THC; 3,4-metilenodioximetanfetamina – MDMA; e 3,4-metilenodioxiafetamina - MDA) e F3 (outras substâncias) (BRASIL, 1998).

O mercado de drogas sintéticas é caracterizado por uma variedade de substâncias psicoativas de diferentes classes farmacológicas, como os estimulantes tipo anfetaminas (ATS, do inglês *amphetamine-type stimulants*) e as Novas Substâncias

Psicoativas (NSP), que têm surgido mais frequentemente no mercado nos últimos vinte anos (LEAL CUNHA et al., 2021).

As ATS são substâncias sintéticas que são estruturalmente semelhantes às anfetaminas e metanfetaminas. As anfetaminas com substituintes do tipo "metilenodióxi" no anel aromático, como o MDMA e o MDA, possuem tanto efeitos estimulantes quanto alucinógenos em doses relativamente baixas e são amplamente utilizadas para fins recreativos em boates e em festas de música eletrônica (CARVALHO et al., 2012; KAHL et al., 2021).

O MDMA ou *ecstasy* é uma substância ilícita amplamente consumida, sendo normalmente consumida por via oral, na forma de comprimido. No ano de 2018 foram gerados 587 laudos periciais dessa droga, correspondendo à apreensão de 146.489 comprimidos e 75,44 kg de sólidos (pós ou cristais). Isso representou um significativo aumento tanto no número de laudos quanto no número de apreensões com relação ao ano de 2017, quando foram gerados 142 laudos referentes à apreensão de 20.091 comprimidos e 12,587 kg de sólidos, tornando o MDMA a droga sintética com o maior número de apreensão pela Polícia Federal Brasileira nos anos de 2017 e 2018. Concomitantemente, o MDA foi a 6ª droga sintética com o maior número de laudos no ano de 2017 e alcançou a 3ª posição no ano de 2018. Segundo a Polícia Federal, este aumento de apreensões de MDA pode estar relacionado à atuação de laboratórios que sintetizam esta substância de forma clandestina no país, conforme constatado em operações policiais (POLÍCIA FEDERAL, 2018).

Os efeitos psicoestimulantes do MDMA podem ser observados de 30 a 60 minutos após a ingesta oral, podendo persistir por até 8 horas (KRASOWSKI, 2019).

Assim, é prontamente absorvido pelo trato intestinal e atinge seu pico de concentração no plasma cerca de 2 horas após a administração. A eliminação da droga do corpo é moderadamente lenta, sendo necessário cerca de 40 horas para que aproximadamente 95% da droga seja eliminada do organismo. Produtos de biotranformação como o MDA, formado pelo processo de desmetilação via CYP1A1, são famacologicamente ativos, prolongando a duração dos efeitos desta substância (KALANT, 2001). Os principais efeitos psicoestimulantes do ecstasy incluem a sensação de euforia, estimulação psicomotora, alucinações visuais e auditivas, aumento da socialização, sensibilização ao toque e aumento das respostas emotivas para estímulos considerados normais. Os seus efeitos adversos normalmente são correlacionados a hipertermia, hipertensão, perda da consciência, arritmias e taquicardia (DORTA et al., 2018; DUARTE et al., 2020).

Paralelamente, as chamadas Novas Substâncias Psicoativas (NSP), também conhecidas por *design drugs* representam uma nova ameaça devido ao ritmo acelerado de consumo no Brasil (POLÍCIA FEDERAL, 2018). Estas substâncias sintéticas foram planejadas com a finalidade de substituir drogas clássicas já existentes e que são controladas pela legislação internacional como metanfetamina, MDMA, cocaína, *Cannabis* e dietilamida do ácido lisérgico (LSD), mimetizando ou potencializando seus efeitos. As NSP são produzidas através de pequenas alterações na estrutura molecular destas substâncias, dificultando o trabalho de órgãos de fiscalização que necessitam de um constante aprimoramento tecnológico para monitorar a dinâmica evolução dos compostos sinetizados, assim como de órgãos de saúde pública que desconhecem com que substâncias estão lidando ou os prováveis efeitos tóxicos a longo prazo (ZACCA et al., 2021). De acordo com a *European Monitoring Centre for Drugs and Drugs*

Addiction (EMCDDA), as NSP são substâncias que não foram controladas pela Convensão Única sobre Entorpecentes de 1961 nem pela Convenção sobre Substâncias Psicotrópicas de 1971, mas que são capazes de causar sérios problemas à saúde pública (EMCDDA, 2006).

Estas substâncias tornaram-se um fenômeno global, sendo que 126 países e territórios de todas as regiões do mundo já reportaram ao Escritório das Nações Unidas sobre Drogas e Crime (UNODC) o aparecimento de pelo menos uma NSP em seus territórios até o ano de 2020, representando um total de 1047 substâncias identificadas (UNODC, 2021). Desta forma, observa-se que a distribuição global de NSP continua a ser caracterizada pela emergência de um grande número de novas substâncias, pertencentes a diversos grupos químicos. Normalmente utiliza-se um sistema de listagem nominal para classificar as substâncias sob controle. Porém, na tentativa de acompanhar o crescente aparecimento das NSP, alguns países alteraram a forma de atualização das listas, aderindo a formas de classificação com abordagens mais genéricas. No sistema genérico é estabelecida a estrutura molecular nuclear e possíveis variações particulares desta estrutura que devem ser controladas, antecipando o controle sobre novas substâncias que, porventura, venham a ser utilizadas (ANVISA, 2019).

A categorização oficial das NSP estabelece nove classes de substâncias, sendo estas: triptaminas, catinonas sintéticas, canabinoides sintéticos, substâncias vegetais, piperazinas, feniletilaminas, substâncias do tipo fenciclidina, aminoindanos e outras substâncias, que podem incluir compostos como benzodiazepínicos sintéticos. Além disso, estas substâncias são categorizadas de acordo com os seus efeitos, nas seguintes categorias: estimulantes, opioides, canabinoides, dissociativos, alucinógenos e sedativos (UNODC, 2019). Conforme relatório da Polícia Federal (PF), elaborado com base na

análise dos Laudos emitidos pelas unidades de Criminalística da Polícia Federal de todo o Brasil no ano de 2018, as classes de NSP predominantes nas apreensões foram as catinonas sintéticas e as feniletilaminas, representando 66,8% e 15,7% das apreensões, respectivamente (POLÍCIA FEDERAL, 2018).

A feniletilamina é a estrutura-base de diversos compostos, como anfetaminas, catinonas e os chamados de 2C. O termo 2C é um nome geral para o grupo de fenetilaminas psicodélicas que contém em comum a estrutura da fenetilamina com dois grupamentos metóxi nas posições 2 e 5 do anel aromático e diferentes substituintes na posição 4 e, raramente, na posição 3 (ZUBA; SEKUŁA; BUCZEK, 2012). Nos últimos anos, um grupo de derivados tóxicos das fenetilaminas da classe 2C, denominado NBOMe, tornou-se popular pelas vendas na *internet* (DA CUNHA; EBERLIN; COSTA, 2018; WAYHS et al., 2016). Os NBOMes produzem efeitos alucinógenos, uma vez que atuam como agonistas parciais ou totais dos receptores serotoninérgicos 5-HT_{2A}, sendo consumidos majoritariamente por via oral ou sublingual e esporadicamente por via intravenosa, nasal, vaginal e retal (BERSANI et al., 2014). Em razão do menor custo, maior disponibilidade e pelo fato de alguns NBOMes ainda não serem considerados proscritos em alguns países, o uso destas substâncias vem substituindo gradativamente o consumo de alucinógenos psicodelicos como o LSD, dimetiltriptamina e mescalina (DA CUNHA; EBERLIN; COSTA, 2018).

A síntese dos NBOMes ocorre a partir da adição do grupamento N-metoxibenzil ao composto análogo 2C-X, o que aumenta a afinidade do composto com o receptor 5-HT_{2A}. Por exemplo, o 25I-NBOMe apresenta uma afinidade 10 vezes maior com este receptor do que o análogo 2C-I. Por esse motivo, concentrações diminutas, na ordem de

microgramas, são capazes de produzir efeitos no organismo (LAWN et al., 2014). A **Figura 1** apresenta um comparativo entre a estrutura química destes compostos.

Figura 1 Comparação entre as estruturas do 2C-I (1) e o 25I-NBOMe (2).

(1) **(2)**

Os principais efeitos psicoestimulantes relatados por indivíduos que consumiram estas substâncias são a euforia, alucinações visuais, estimulação física, sentimento de amor e empatia, mudança na consciência e sensações corporais inusitadas. Já os efeitos adversos podem incluir a confusão, agitação, náuseas, insônia, paranoia, problemas de memória de curto prazo, mudanças de humor, hipertensão, arritmias e taquicardia (KUEPPERS; COOKE, 2015; ZAWILSKA; ANDRZEJCZAK, 2015).

As substâncias selecionadas para serem estudadas neste trabalho foram as drogas sintéticas MDA, MDMA, 25B-NBOMe, 25C-NBOMe e 25I-NBOMe, cujas estruturas químicas, massas molares e algumas propriedades físico-químicas estão dispostas na **Tabela 1**.

Tabela 1 Compostos estudados com suas respectivas estruturas químicas e valores de pKa e de logP.

Substância	Estrutura Química	Massa molar (g mol^{-1})	pKa	logP
MDA		179,22[a]	9,67[a]	1,6[a]
MDMA		193,24[a]	9,9[a]	2,2[a]
25B-NBOMe		380,30[a]	8,88[b]	3,9[a]
25C-NBOMe		335,80[a]	8,88[b]	3,8[a]

Substância	Estrutura Química	Massa molar (g mol^{-1})	pKa	logP
25I-NBOMe		427,30[a]	8,91[b]	3,8[a]

Fonte: [a] (PUBCHEM, 2020); [b] (CHEMICALBOOK)

A característica de solubilidade em lipídios é expressa pelo coeficiente de partição octanol/água (logP), sendo que a velocidade de passagem através das membranas está correlacionada com esta propriedade. Quanto mais lipossolúvel o composto maior será o seu logP, mais fácil será a sua passagem pela barreira hematoencefálica e, consequentemente, mais rápido será o acesso ao Sistema Nervoso Central (DORTA et al., 2018).

De uma forma geral, as moléculas não ionizadas são mais lipossolúveis e podem difundir-se mais facilmente pela membrana celular. Já as moléculas ionizadas são menos capazes de penetrar na membrana lipídica, uma vez que são pouco lipossolúveis e sua transferência depende da permeabilidade da membrana, que é determinada por sua resistência elétrica. O pKa é o pH no qual a metade da substância (ácido ou base fraca) está em sua forma ionizada, apresentando influencia na distribuição transmembrana de um eletrólito fraco (BRUNTON, 2018).

Os compostos estudados são básicos e quando em solução dissociam-se em fração ionizada e não ionizada, em uma proporção que depende do pH do meio e do pKa. Quando em contato com um meio ácido como o estomacal, apresentam elevado grau de ionização, resultando em baixa absorção. No entanto, em meios mais básicos como no intestino, ocorre o predomínio da forma não ionizada, proporcionando elevada absorção (LEVINE, 2003).

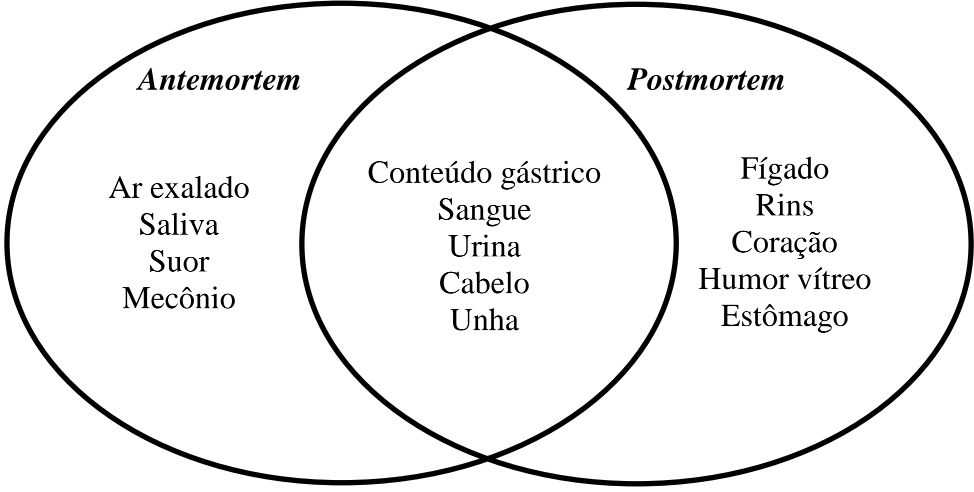
1.2 ANÁLISES TOXICOLÓGICAS *POSTMORTEM*

A toxicologia forense é uma ciência de caráter historicamente analítico que constatemente é utilizada para o auxílio às questões em âmbito judicial, sendo as três principais áreas de aplicação desta ciência a análise *postmortem*, a testagem de drogas de abuso e a toxicologia do desempenho humano. Neste cenário, as amostras de interesse, geralmente, estão restritas às amostras biológicas. Há uma grande diversidade de amostras que podem ser utilizadas em análises forenses, sendo que a escolha da matriz biológica a ser utilizada deve levar em consideração a disponibilidade da amostra e o tipo de exposição. Nos casos de exposição aguda, como dopagem no esporte e tentiva de suicídio, as matrizes mais adequadas são sangue, urina e saliva. Já em casos de exposição crônica, como, por exemplo, em casos de envenenamento por arsênio, as matrizes mais adequadas são cabelos e unhas, uma vez que fornecem informações relativas a um longo período de exposição (BORDIN et al., 2015; DORTA et al., 2018; DRUMMER, 2004).

A coleta de amostras na toxicologia forense pode ser realizada em indivíduos vivos (*antemortem*) ou mortos (*postmortem*). A **Figura 2** apresenta as principais

amostras utilizadas nas análises toxicologicas forense, sendo que estas estão segregadas conforme o procedimento de coleta.

Figura 2 Principais amostras utilizadas em toxicologia forense.



Fonte: Adaptado de (DORTA et al., 2018)

Entre as principais matrizes utilizadas nas análises toxicológicas forense, destaca-se o sangue por fornecer a apropriada correlação entre a concentração das substâncias e o estado clínico do indivíduo (NIU et al., 2018). Esta matriz biológica representa um fluido complexo, levemente alcalino (pH 7,4), constituído em grande parte de água (cerca de 80%), proteínas solúveis, gorduras, sais e células suspensas. Análises quantitativas em amostras de sangue podem fornecer importantes informações para diferenciar intoxicações agudas de intoxicações crônicas, através das concentrações de droga inalterada e dos respectivos produtos de biotransformação. Em casos de introxicações agudas, geralmente as concentrações da droga inalterada são consideravelmente maiores do que a concentração dos produtos de biotransformação, enquanto que em casos de intoxicações crônicas o inverso é observado (DORTA et al., 2018).

Apesar das vantagens, as amostras de sangue representam um maior desafio do ponto de vista analítico quando comparadas às amostras de urina, em função da composição mais complexa. Além disso, as concentrações das substâncias encontradas no sangue são baixas, em nível traço, sendo estas consideravelmente inferiores àquelas encontradas na urina. Com relação à janela de detecção, as amostras de sangue podem ser utilizadas para fornecer informações sobre a utilização de drogas em um curto espaço de tempo, indicando o consumo realizado há poucos dias. Outra desvantagem em se trabalhar com amostras de sangue está no fato da coleta ser realizada através de um procedimento invasivo, o que acarreta maior risco de contaminação e exige a atuação de profissionais treinados para realiza-la (FLANAGAN; CONNALLY; EVANS, 2005; SILVA et al., 2018).

As amostras de sangue podem ser avaliadas na forma de sangue total, plasma – que é o sobrenadante obtido através da centrifugação do sangue adicionado de anticoagulante - ou soro - que é o constituído pelo plasma sem os fatores de coagulação, podendo ser obtido pela centrifugação do sangue coagulado (BORDIN et al., 2015). O sangue coletado em casos *postmortem* difere consideravelmente daquele coletado de pessoas vivas, sendo que, muitas vezes, a obtenção de soro ou plasma a partir deste tipo de amostra torna-se inviável. Em função disto, os métodos em toxicologia forense utilizam, geralmente, amostras de sangue total (DRUMMER, 2007; SÁNCHEZ DE LA TORRE; MARTÍNEZ; ALMARZA, 2005). A **Tabela 2** apresenta as concentrações das substâncias estudadas neste trabalho em amostras de sangue total em casos de intoxicações fatais reportadas na literatura.

Tabela 2 Concentrações dos analitos em amostras de sangue total em casos fatais de intoxicações reportadas na literatura.

Analitos	Coleta da amostra	Concentração (ng mL^{-1})	Referência
MDMA and MDA	*postmortem*	Caso 1: 170 (MDMA); 30 (MDA) Caso 2: 1090 (MDMA); 80 (MDA) Caso 3: 40 (MDMA)	(GARCÍA-REPETTO et al., 2003)
	postmortem	Caso 2: 1971,5 (MDMA); 204,2 (MDA)	(VAIANO et al., 2016)
MDMA	*postmortem*	Caso 7: 40 Caso 8: 90	(FERRARI JÚNIOR; CALDAS, 2018)
25B-NBOMe	*postmortem*	10	(CHAN; WU; LEE, 2019)
	postmortem	1,59 (sangue do coração)	(SHANKS; SOZIO; BEHONICK, 2015)
25I-NBOMe	*antemortem*	0,76	(LOWE; PETERSON; COUPER, 2015)
	postmortem	0,405 (sangue periférico) 0,410 (sangue do coração)	(POKLIS et al., 2014)
	postmortem	19,8 (sangue do coração)	(SHANKS; SOZIO; BEHONICK, 2015)
	postmortem	28	(KUEPPERS; COOKE, 2015)
	antemortem	0,25	(UMEMURA et al., 2015)
25C-NBOMe	*antemortem*	0,81	(ANDREASEN et al., 2015)
	postmortem	0,60	(ANDREASEN et al., 2015)
	postmortem	Sangue do coração: 1,43	(MORINI et al., 2017)

Fonte: autoria própria

Conforme os dados apresentados na **Tabela 2,** as concentrações reportadas variaram de 40 a 1971,5 ng mL^{-1} para MDMA; 30 a 204,2 ng mL^{-1} para MDA; 1,59 a 10 ng mL^{-1} para o 25B-NBOMe; 0,25 a 19,8 ng mL^{-1} para o 25I-NBOMe; e 0,6 a 28 ng mL^{-1} para o 25C-NBOMe. Sendo assim, é possível constatar que as concentrações de NBOMes em amostras de sangue reportadas para casos fatais são consideravelmente inferiores às concentrações de MDMA e MDA.

As análises toxicológicas *postmortem* apresentam diversos desafios, como a implementação de metodologias analíticas capazes de identificar e, muitas vezes, quantificar substâncias envolvidas em um provável caso de intoxicação, devido à complexidade da composição das amostras, que podem conter interferentes e incompatibilidades com os instrumentos analíticos, além das baixas concentrações dos analítos (MERIB et al., 2018). A fim de contornar tais dificuldades e realizar a determinação adequada das substâncias de interesse, diversas técnicas de preparo de amostras foram e estão sendo desenvolvidas.

1.3 TÉCNICAS DE PREPARO DE AMOSTRAS

Os principais objetivos do preparo de amostra são reduzir ou eliminar possíveis interferentes da matriz, isolar os analitos, concentrá-los para análise de traços e tornar possível a introdução da amostra no equipamento que fornecerá os dados analíticos. A etapa de preparo de amostras é de fundamental importância e é considerada, muitas vezes, a mais crítica de uma análise química, em função do risco de perda de analitos, contaminação da amostra e por ser a etapa mais demorada dos procedimentos analíticos (CARASEK; MERIB, 2015; NIU et al., 2018). A escolha da técnica de preparo de amostras deve ser fundamentada na compreensão dos princípios que governam a

transferência de massa dos analitos em sistemas multifásico, sendo que esta é baseada em características dos componentes da matriz, tais como polaridade, solubilidade, estabilidade química e térmica, coeficiente de partição, lipofilicidade, entre outros (BORGES, K. B.; FIGUEIREDO, E. C.; QUEIROZ, 2015; PAWLISZYN, 2012).

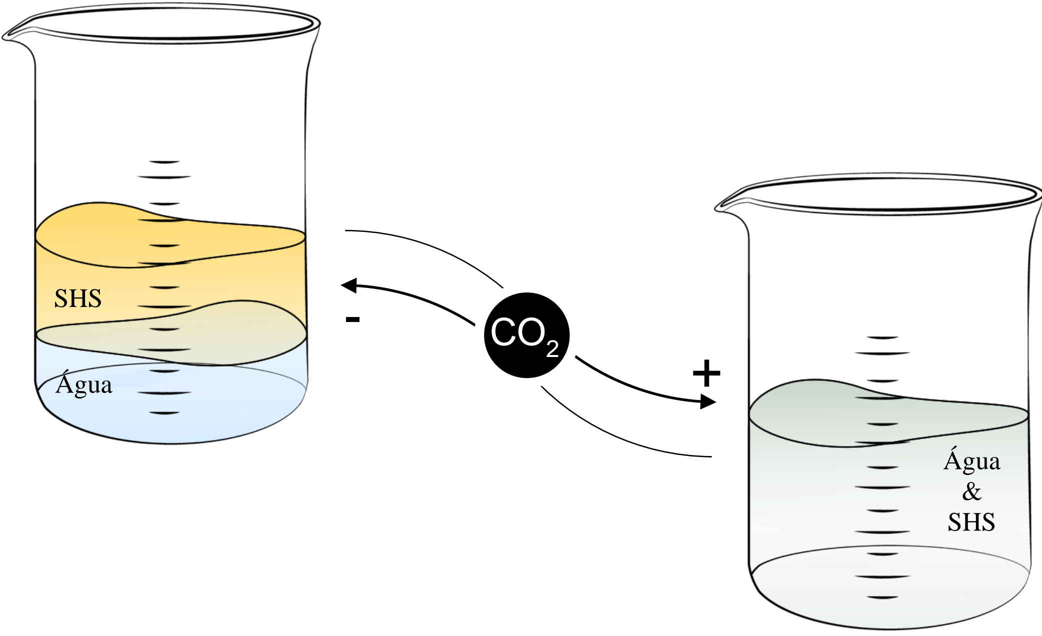
Neste contexto, existem técnicas clássicas de preparo de amostras que são bastante conhecidas e amplamente utilizadas nas analises toxicológicas, tais como a extração líquido-líquido (LLE, do inglês *liquid-liquid extraction*) e a extração em fase sólida (SPE, so inglês *solid-liquid extraction*). Essas técnicas apresentam alguns pontos negativos, como o uso de grandes volumes de solventes orgânicos tóxicos, longo tempo de extração e grande geração de resíduos. Considerando a preocupação mundial com a sustentabilidade, a química analítica moderna tem sido direcionada para a simplificação e miniaturização dos processos, seguindo os princípios da química verde (CARASEK; MORÉS; MERIB, 2018; MARTINS et al., 2021). As técnicas de microextração são divididas em técnicas de microextração em fase sólida, na qual se destacam a microextração em fase sólida (SPME, do inglês *solid phase microextraction*), a microextração em barra adsortiva (BAµE, do inglês *bar adsorptive microextraction*) e a extração em ponteiras descartáveis (DPX, do inglês *disposable pipette extraction*); e em técnicas de microextração em fase líquida, que engloba a microextração em gota única (SDME, do inglês *single-drop microextraction*), a microextração líquido-líquido dispersiva (DLLME, do inglês dispersive *liquid-liquid microextraction*) e a microextração líquido-líquido homogênea (HLLME, do inglês *homogeneous liquid-liquid microextraction*) (CARASEK; MORÉS; MERIB, 2018; CORAZZA et al., 2020; SOARES DA SILVA BURATO et al., 2020). Neste trabalho foi utilizada a técnica de

HLLME realizada como os usos solventes de hidrofilicidade comutável (SHSs, do inglês *switchable hydrophilicity solvents*).

A HLLME é uma técnica de preparo baseada no processo de separação de fases a partir de uma solução homogênea, sendo que ela compreende duas etapas. Primeiramente é realizada a completa miscibilidade da amostra e da fase orgânica, o que fornece uma superfície de contato infinitamente grande para a extração, facilitando o processo de extração. A solubilização do solvente orgânico é modulada por suas propriedades químicas ou através da utilização de energia externa, como aquecimento ou ultrassom. Posteriormente é realizada a separação das fases mediante a adição ou remoção de um reagente específico, o que possibilita a transferência do analito da solução homogênea para um pequeno volume de solvente. Esta separação de fases geralmente é realizada através do efeito *salting out*, mudanças de pH ou par iônico (AHMAR et al., 2018; LASARTE-ARAGONÉS et al., 2015).

Nos últimos anos, uma nova classe de solventes foi proposta para atuar como solvente extrator nessa técnica de microextração, denominados de SHSs. Para um composto ser considerado um SHS, ele deve ser capaz de comutar entre uma forma miscível e uma forma imiscível em solução aquosa, dependendo do pH do meio. Essa alternância entre as formas hidrofílicas e hidrofóbicas possibilita a separação de fases de maneira simples e favorece a criação de um promissor campo para o desenvolvimento de procedimentos de microextração para a determinação de diversos tipos de substâncias (CHERKASHINA et al., 2019). A **Figura 3** ilustra o comportamento dos SHSs com a mudança de pH realizada através da adição e remoção de CO_2.

Figura 3 Comportamento de um solvente de hidrofilicidade comutável quando ocorre a adição ou remoção de CO_2



Fonte: autoria própria

Conforme o apresentado na **Figura 3,** inicialmente o SHS está na sua forma hidrofóbica, ou seja, imiscível em água; com a adição de CO_2, o SHS passa para a sua forma hidrofílica, tornando-se solúvel em água e formando uma solução monofásica. Com a remoção do CO_2 do sistema, feita através de aquecimento ou introdução de um gás inerte, o SHS retorna para a sua forma hidrofóbica, formando uma solução bifásica novamente. As aminas terciárias, aminas secundárias, aminas com grupo funcional incorporado, amidas e ácidos graxos podem possuir características de hidrofilicidade comutável (JESSOP et al., 2010; VANDERVEEN; DURELLE; JESSOP, 2014).

Os SHSs possuem diversas vantagens com relação aos solventes geralmente empregados em técnicas de extração, como a menor toxicidade, baixo custo e o fato de possibilitarem a realização de extrações rápidas e eficientes. Além disso, a mudança de pH para a comutação é realizada de forma fácil através da adição e remoção de CO_2,

como mostrado anteriormente, ou através da adição de um reagente específico, como uma solução concentrada de hidróxido de sódio (BAZEL; REČLO; CHUBIRKA, 2020). A **Tabela 3** apresenta um levantamento da aplicação dos SHSs em matrizes biológicas reportados na literatura.

Tabela 3 Utilização de solventes de hidrofilicidade comutável em técnicas de microextração para o preparo de amostras biológicas reportadas na literatura.

SHS	**Matriz**	**Extração**	**Analito**	**Instrumentação Analítica**	**Referência**
DMCHA	Urina	µP-SHS-HLLME	Antidepressivos	GC-MS	(LUIZ OENNING et al., 2020)
EHPA	Urina	SHS-BME	Antimicrobianos	HPLC-UV	(POCHIVALOV et al., 2020)
OA	Urina	SS-LPME-SFO	Hidrocarbonetos Policíclicos Aromáticos	HPLC-FLD	(WANG et al., 2020)
DMBA	Plasma	SS-LPME	Metionina	GC-MS	(ERARPAT et al., 2020)
DMCHA	Urina, leite e saliva	SHS-LLME	Anti-inflamatórios não esteroidais	HPLC-DAD	(HASSAN; ALSHANA, 2019)
DPA	Urina	SS-HLLME	Nitrazepam	DPV	(SHAHRAKI; AHMAR; NEJATI-YAZDINEJAD, 2018)
DPA	Urina	SHS-HLLME	Metanfetamina	GC-MS	(SHAHVANDI; BANITABA; AHMAR, 2018)
DPA	Urina	SHS-HLLME	Metadona e Tramadol	GC-FID	(AHMAR et al., 2018)
DPA	Soro e leite	SHS-HLLME	Antidepressivos	GC-FID	(BEHPOUR et al., 2020)

SHS	Matriz	Extração	Analito	Instrumentação Analítica	Referência
TEA	Urina e Plasma	IP-SHS-HLLME	Paraquat	HPLC-UV	(RAHIMI KAKAVANDI et al., 2017)
HexA	Urina	EA-SS-LPME	Ofloxacina	HPLC-FLD	(VAKH et al., 2016)
TEA	Cabelo	SHS-LPME	Chumbo e Cadmio	GFAAS	(ZHANG et al., 2018)
TEA	Cabelo	SS-LPME	Cobre	FAAS	(YILMAZ; SOYLAK, 2015)
DMCHA	Cabelo	SHS-LPME	Mercúrio	UV/VIS	(KHAN; SOYLAK, 2016)
TEA	Urina	SS-LPME	Cádmio, níquel, chumbo e cobalto	FAAS	(HABIBIYAN et al., 2017)
TEA	Urina e plasma	SIP-SS-DLLME	Fenazopiridina	UV/VIS	(HAMID; FAT'HI, 2018)
DBU e decanol	Sangue	SHS-LPME	Alumínio	FAAS	(ARAIN et al., 2018)
DBU	Plasma	SS-LPME	Aminoácidos	HPLC-FLD	(ZARE et al., 2018)
DMCHA	Urina	SHS-LLME	Drogas (meperidina, cetamina, tramadol, diazepam, papaverina,	GC-MS	(XU et al., 2018)

SHS	Matriz	Extração	Analito	Instrumentação Analítica	Referência
			clozapina, zolpidem, metaqualona, clordiazepoxide)		
HexA	Urina	SM-LPME	Tetraciclina	HPLC-UV	(LEBEDINETS et al., 2020)

Fonte: autoria própria.

DMCHA: N,N-dimetilciclohexilamina;
EHPA: Ácido di(2-etil-hexil) fosfórico;
AO: Ácido Octanóico;
DMBA: N,N-dimetilbenzilamina;
DPA: Dipropilamina;
TEA: Trietilamina;
HexA: Ácido Hexanóico;
DBU: 1,8-Diazabicyclo [5.4.0] undec-7-eno.

µP-SHS-HLLME: Microextração Líquido-Líquido Homogênea com Solvente de Hidrofilicidade Comutável em µ-Pipeta;
SHS-BME: Microextração baseada em Solvente com Hidrofilicidade Comutável;
SS-LPME-SFO: Microextração em Fase Líquida com Solvente Comutável com Solidificação de Gotas Orgânicas Flutuantes;
SS-LPME: Microextração em Fase Líquida com Solvente Comutável;
SHS-LLME: Microextração Líquido-Líquido com Solvente de Hidrofilicidade Comutável;
SS-HLLME: Microextração Líquido-Líquido Homogênea com Solvente Comutável;
SHS-HLLME: Microextração Líquido-Líquido Homogênea com Solvente de Hidrofilicidade Comutável;
IP-SHS-HLLME: Microextração Líquido-Líquido Homogênea com Solvente de Hidrofilicidade Comutável e Par Iônico;
EA-SS-LPME: Microextração em Fase Líquida baseada em Solvente Comutável Assistida por Efervescência;
SHS-LPME: Microextração em Fase Líquida com Solvente de Hidrofilicidade Comutável;
SS-LPME: Microextração em Fase Líquida com Solvente Comutável;
SIP-SS-DLLME: Microextração Líquido-Líquido Homogênea com Solvente de Hidrofilicidade Comutável e Par Iônico Surfactante;
SHS-LLME: Microextração Líquido-Líquido com Solvente de Hidrofilicidade Comutável;
SM-LPME: Microextração em Fase Líquida com Membrana por Agitação.

GC-MS: Cromatografia Gasosa acoplada à Espectrometria de Massas;
HPLC-UV: Cromatografia Líquida de Alta Eficiência acoplada ao Detector de Ultravioleta;
HPLC-FLD: Cromatografia Líquida de Alta Eficiência acoplada ao Detector de Fluorescência;

HPLC-DAD: Cromatografia Líquida de Alta Eficiência acoplada ao Detector de Arranjo de Diodos;
DPV: Voltametria de Pulso Diferencial; GC-FID: Cromatografia Gasosa com Detector por Ionização de Chama;
GFAAS: Espectrometria de Absorção Atômica com forno de grafite;
FAAS: Espectrometria de Absorção Atômica com chama;
UV/VIS: Espectrometria no Ultravioleta-visível.

Analisando a **Tabela 3**, observa-se que a matriz biológica mais utilizadas em microextrações com SHSs é a urina. Estudos empregando outras matrizes biológicas como leite materno, saliva, cabelo, soro, plasma e sangue total são relatados, entretanto, a aplicação nas análises toxicológicas permanece pouco explorada. Com relação a etapa instrumental, a análise é realizada, geralmente através de métodos de separação que incluem a cromatografia líquida e gasosa, sendo que há alguns trabalhos envolvendo técnicas espectrométricas e técnicas voltamétricas. Adicionamente, é possível verificar a incipiente construção de metodologias de espectrometria de massas, sendo essa técnica analítica majoritarimanete acoplada a cromatografia gasosa (GC-MS) para determinação de compostos extraídos com SHSs. De acordo com nosso conhecimento, não foram relatos até o momento estudos utilizando a cromatografia líquida acoplada a espectrometria de massas (LC-MS) para este propósito, possibilitando assim um campo novo a ser explorado, visto que essa técnica analítica apresenta elevada seletividade, fornecendo baixos limites de detecção e quantificação, fator indispensável para a aplicação nas análises toxicológicas forense, principalmente considerando substâncias altamente potentes e potencialmente letais em baixas concentrações.

Os SHSs escolhidos para serem utilizados neste trabalho foram a amina secundária dipropilamina (DPA) e a amina terciária N,N-dimetilciclohexilamina (DMCHA) por serem compostos básicos como as substâncias estudadas e por apresentarem logP próximo aos dos analitos, facilitando o processo de extração. As estruturas químicas, valores de pKa e logP dos SHSs utilizados estão dispostos na **Tabela 4**.

Tabela 4 SHSs empregados neste trabalho com suas respectivas estruturas químicas e valores de pKa e de logP.

Solvente	**Estrutura Química**	**pKa**	**logP**
Dipropilamina (DPA)		10,77	1,57
N,N-dimetilciclohexilamina (DMCHA)		10,22	1,99

Fonte: (CHEMICALIZE, 2021)

Diante do exposto, torna-se relevante desenvolver uma metodologia rápida, eficiente, fácil, sensível, de baixo custo e com pouca geração de resíduos ao ambiente, capaz de detectar diferentes substâncias psicoativas, a fim de aprimorar o monitoramento da exposição a estes compostos. Neste trabalho foi proposto um método empregando SHS como solvente extrator na técnica de HLLME para a extração de MDMA, MDA e NBOMes (25B, 25C e 25I) em amostras de sangue *postmortem* e determinação por cromatografia líquida acoplada à espectrometria de massas. Cabe ressaltar que, até o momento, não há trabalhos publicados utilizando esta técnica de extração para a determinação destas drogas de abuso. O trabalho desenvolvido foi submetido no periódico *Journal of Analytical Toxicology*.

2. OBJETIVO

2.1 OBJETIVO GERAL

Desenvolvimento e validação de metodologia analítica para a determinação das drogas sintéticas MDMA, MDA e NBOMes (25B, 25C e 25I) em amostras de sangue empregando microextração líquido-líquido homogênea com solventes de hidrofilicidade comutável (SHS-HLLME) e determinação por cromatografia líquida acoplada à espectrometria de massas (LC-MS/MS).

2.2 OBJETIVOS ESPECÍFICOS

- Otimização dos parâmetros cromatográficos de separação e do espectrômetro de massas;
- Otimização das principais variáveis da etapa de extração, tais como tipo de solvente de hidrofilicidade comutável (SHS), volume da mistura SHS:HCl 6M, volume de amostra, volume de hidróxido de sódio e tempo de extração;
- Determinação dos parâmetros de validação conforme guia do Grupo de Trabalho Científico de Toxicologia Forense (SWGTOX);
- Aplicação do método desenvolvido em amostras provenientes do Departamento de Perícias Laboratoriais do Instituto Geral de Perícias (DPL-IGP) do Rio Grande do Sul.

3. MANUSCRITO

O manuscrito **Application of homogenous liquid-liquid microextraction with switchable hydrophilicity solventes to the determination of MDMA, MDA and NBOMes in post-mortem blood samples**, que conta com a parte experimental desenvolvida nesta dissertação, foi submetido para publicação na revista *Journal of Analytical Toxicology* (ISSN *Online* 1945-2403, fator de impacto 3.513, 2019) e está apresentado a seguir. As normas de publicação estão disponíveis no Anexo II.

1 **Application of homogeneous liquid-liquid microextraction with switchable**
2 **hydrophilicity solvents to the determination of MDMA, MDA and NBOMes in**
3 **postmortem blood samples**

4

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20 **Abstract**

21 Synthetic drugs for recreational purposes are in constant evolution and their
22 consumption promote a significant increase in intoxication cases, resulting in damaging
23 public health. The development of analytical methodologies to confirm the consumption
24 of illicit drugs in biological matrices are required for control of these substances. This
25 work exploited the development of an extraction method based on homogenous liquid-
26 liquid microextraction with switchable hydrophilicity solvent as extraction phase (SHS-
27 HLLME) for the determination of the synthetic drugs MDMA, MDA and NBOMes
28 (25B, 25C and 25I) in postmortem blood, followed by liquid chromatography coupled
29 to mass spectrometry in tandem (LC–MS/MS). The optimized sample preparation
30 conditions consisted of using 250 µL of $ZnSO_4$ 10% and 50 µL of NaOH 1 mol/L in the
31 protein precipitation step; *N,N*-dimethylcyclohexylamine (DMCHA) was used as
32 switchable hydrophilicity solvent (SHS), 650 µL of a mixture of SHS:HCl 6 mol/L (1:1
33 v/v), 500 µL of whole blood, 500 µL of NaOH 10 mol/L and 1 min of extraction time.
34 The proposed method was validated, providing determination coefficients higher than
35 0.99 for all analytes; LOD and LOQ ranged from 0.1 to 10 ng/mL; intra-run precision
36 from 2.16 to 9.19%; inter-run precision from 2.39 to 9.59%; bias from 93.57 to
37 115.71%; and matrix effects from 28.94 to 51.54%. The developed method was
38 successfully applied to four authentic postmortem blood samples from synthetic drugs
39 users, and it was found to be reliable with good selectivity.

40

41 ***Keywords*****:** Substituted phenethylamines; MDMA; MDA; NBOMes; switchable
42 hydrophilicity solvent.

Introduction

Psychoactive substance consumption is considered a worldwide major public health issue, involving a variety of health and social consequences (1). The synthetic drug market is characterized by a variety of psychoactive substances of different chemical and pharmacological classes, such as substituted phenethylamines (2,3). Amphetamine and its methylenedioxy-derivatives like 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxyethylamphetamine (MDEA) are widely used for recreational purposes and acute psychophysiological effects include euphoria, psychomotor stimulation, visual and auditory hallucinations, increased socialization, increased emotional responses, hyperthermia, hypertension, loss of consciousness, arrhythmias, and tachycardia (4-6).

NBOMes are a class of new psychoactive substances (NPS) with hallucinogenic effects similar to lysergic acid diethylamine. These compounds are derived from 2C-X phenethylamines with addition of halogens or chemical groups that can substantially increase their hallucinogenic potential, acting as potent agonists of serotonin 5-HT_{2A} receptors (7). A increasing of number of intoxications by substituted phenethylamines have been reported in the last years (8-18). Generally, the consumption of low amount of these compounds are sufficient to produce psychoactive and hallucinogenic effects. Thus, the identification in biological matrices requires efficient extraction procedures and highly sensitive detection (19,20).

The development of analytical strategies consists of a significant challenge due to the high complexity of biological matrices require a sample preparation step prior to

66 instrumental analysis due to the presence of proteins, salts and other organic compounds
67 (20). Sample preparation step can include the removal of interfering substances,
68 conversion of the analytes into a more suitable form before the analysis and
69 preconcentration of the analytes (21). In this scenario, microextraction techniques have
70 gaining attention for NPS determination.

71 Homogenous liquid-liquid microextraction (HLLME) is a powerful sample
72 preparation technique that can reduce the extraction time and costs of analysis;
73 moreover, the consumption and exposure to organic solvents are also significantly
74 reduced. Firstly, this approach involves the complete solubilization of the extraction
75 solvent in aqueous sample making the contact surface between phases infinitely large.
76 After, the two phases are separated by the addition or removal of a particular reagent
77 (22). Recently, microextraction techniques have been performed with green solvents to
78 reduce waste and to eliminate toxic solvents used in analytical procedures. In this
79 context, switchable hydrophilicity solvents (SHSs) are considered green possibilities to
80 be used as extraction phase in HLLME-based techniques (23). SHSs are organic bases
81 or acids such as amines and amidines. These solvents exhibit the feature of switching
82 between the hydrophilic and hydrophobic forms in aqueous solutions by changing
83 sample pH, that can be performed by adding and removing CO_2 or adding a specific
84 reagent, such as NaOH (24,25).

85 SHSs have been employed for the determination of a number of analytes in
86 biological matrices such as methamphetamine (24), antidepressants (26), methadone
87 and tramadol (27), antimicrobial drugs (28), nitrazepam (29), and polycyclic aromatic
88 hydrocarbon monohydroxy metabolites (30) in urine; amino acids (31), and L-

89 methionine (32) in plasm; paraquat in plasm and urine (33); and non-steroidal anti-
90 inflammatory drugs in plasm, urine and saliva (34).

91 The aim of this study was the development, optimization and validation of a
92 method for the determination of substituted phenethylamines (MDA, MDMA, 25C-
93 NBOMe, 25B- NBOMe, and 25I- NBOMe) in postmortem blood samples, using
94 switchable hydrophilicity solvents coupled to LC-MS/MS. The method proposed
95 consists of an environmentally friendly alternative to identify and quantify these
96 analytes in samples suspected of intoxications in forensic toxicology scenario.

97 Experimental

98 Chemical and reagents

99 Analytical standards of MDA, MDMA, 25C-NBOMe, 25B-NBOMe, 25I-NBOMe e
100 MDMA-d_5 (used as internal standard; IS) were obtained from Cerilliant Corporation
101 (Round Rock, USA).

102 Acetonitrile and methanol LC–MS grade were obtained from Merck (Darmstadt,
103 Germany) and formic acid 98% were obtained from Sigma-Aldrich (Milwaukee, USA).
104 The switchable hydrophilicity solvents *N,N*-dimethylcyclohexylamine and
105 dipropylamine were obtained from Sigma-Aldrich (Milwaukee, WI, USA). Sodium
106 hydroxide were purchased from Merck (Darmstadt, Germany) and aqueous solutions of
107 1 mol/L and 10 mol/L were prepared. Hydrochloric acid and zinc sulphate heptahydrate
108 were obtained from Merck (Darmstadt, Germany) and aqueous solutions of 6 mol/L and
109 10% (w/v) were prepared, respectively. Ultrapure water was supplied by a Milli-Q
110 system from Millipore (Billerica, USA).

111 **Preparation of standard solutions**

112 Analytical standards were obtained at concentration of 1 mg/mL in methanol and 100
113 µg/mL in methanol for the deuterated internal standard. Stock solutions of analytes were
114 prepared by dilution of analytical standards in ultrapure water. IS solution was prepared
115 by appropriate dilution of MDMA-d_5 in ultrapure water at 125 ng/mL. All solutions
116 were stored at -20 °C.

117

118 **LC–MS/MS conditions**

119 The analyzes were performed in a UFLC chromatographic system coupled to a triple
120 quadrupole mass spectrometer, model LCMS-8040 (Shimadzu, Kyoto, Japan).
121 Acquity® UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 µm i.d.) maintained at 50 °C
122 was used. The mobile phase consisted of ultra-pure water (A) and acetonitrile (B), both
123 supplemented with formic acid (0.1%, v/v). The following gradient was applied: 0 – 0.5
124 min, 5% of B; 0.5 – 3.5 min, 5 - 90% of B; 3.5 – 3.8 min, 90% of B; 3.8 – 3.9 min, 90 –
125 5% of B; 3.9 – 6.25 min, 5% of B. The mobile phase flow rate was 0.4 mL/min. The
126 mass spectrometer was equipped with electrospray ionization source performed in
127 positive ionization mode. The instrumental parameters were set as follows: heat block
128 temperature 500 °C; capillary voltage, 4.5 kV; nebulizer gas (N_2) flow, 2 L/min;
129 desolvation line temperature, 250 °C; drying gas (N_2) flow, 18 L/min; and collision
130 induced dissociation gas pressure (Ar), 230 kPa. The analyses were performed in
131 multiple reaction monitoring (MRM) mode. Three MRM transitions were chosen for
132 quantification and qualification of each analyte. Table I shows the MRM conditions

133 optimized for the proposed instrumental method. Data treatment was carried out using
134 LabSolutions software (Shimadzu).

135

136 **Postmortem blood samples**

137 Blank samples used for the development and validation of the method were previously
138 analyzed to verify the presence of psychoactive substances according to Fagiolo et al.
139 (35). The application of the method proposed was assessed in four postmortem blood
140 samples from individuals who were suspected to have consumed psychoactive
141 substances, obtained by the Forensic Department of Rio Grande do Sul State Police
142 (*Instituto Geral de Perícias*, IGP-RS, Porto Alegre, RS, Brazil).

143

144 **Preparation of the extraction solvent**

145 The SHS was mixed with hydrochloric acid 6 mol/L (1:1 ;v/v) and a homogeneous
146 solution was observed. Mixtures of SHS:HCl 6 mol/L (1:1, v/v) were prepared,
147 separately, with dipropylamine (DPA) and with *N,N*-dimethylcyclohexylamine
148 (DMCHA) to be used in the optimization step.

149

150 **Optimization of the sample preparation procedure**

151 The variables that can influence on the extraction efficiency of the target compounds
152 were optimized through multivariate strategies. In this step, blank whole blood samples
153 spiked with 5 ng/mL of each analyte were used.

154 Firstly, a fractional factorial design 2^{6-1} was used to assess the influence of some
155 variables on the extraction efficiency. Six variables were evaluated at two levels and a
156 total of 35 experiments were performed, including a triplicate in the central point. The
157 variables included volume of NaOH 1M for the protein precipitation step (10 and 50
158 µL); switchable hydrophilicity solvent type (DPA and DMCHA); volume of the mixture
159 SHS:HCl 6 mol/L (150 and 550 µL); volume of NaOH 10 mol/L (400 and 800 µL); and
160 extraction time (1 and 5 min). After, considering the significant variables, a Doehlert
161 design was performed using volume of SHS:HCl 6 mol/L (500 from 700 µL) and
162 sample volume (350 from 550 µL).

163

164 **Method Validation**

165 The method was validated according to the guideline of Scientific Working Group for
166 Forensic Toxicology (SWGTOX) (36). The analytical parameters evaluated were limits,
167 linearity, precision, bias, carryover, selectivity, matrix effect, dilution integrity and
168 stability.

169 For selectivity evaluation, samples spiked with ephedrine, nicotine, cocaine,
170 sibutramine, sertraline, methamphetamine, and delta-9-THC at concentration of 100
171 ng/mL were subjected to extractions using the method developed.

172 The calibration was performed using the ratio between the analyte peak area and
173 the peak area of the internal standard in *y* axis; and *x* axis corresponded to the analyte
174 concentration. Linearity was performed over 5 runs and calibration curves with six
175 concentrations, added to the postmortem blood samples. Limit of quantitation (LOQ)

176 for each analyte was determined as the first concentration of the linear range which
177 provided satisfactory precision (≤ 20%). Limit of detection (LOD) was established as
178 the value of the lowest non-zero calibrator. Heteroscedasticity was evaluated by
179 application of the *f*-test for each analyte. When presented, weighting factors were used
180 to adjust linearity.

181 Bias and precision (within-run and between-run) were evaluated with 3
182 replicates of each concentration (low, medium, and high) over 5 different runs. Bias
183 was defined as percent target concentration and was required to be within ± 20% of the
184 target concentration; precision accepted was ≤ 20% RSD. Both within-run and between-
185 run was calculated using the one-way analysis of variance (ANOVA) for each QC
186 concentration. Carryover was evaluated by the injection of five extracted blank samples
187 after analyzing the highest concentration of the calibration curve.

188 Matrix effects were determined by spiking two sets of samples at low and high
189 QC concentrations. In set A, 10 blank samples were spiked with all analytes after the
190 extraction. In set B, the same volume of solvent (acetonitrile/water, 15:25, v/v, with
191 0,1% formic acid) only was spiked with analytes (neat sample). Matrix effect was
192 calculated as the peak area ratio of set A to set B, subtracted by 1, and expressed as a
193 percentage; a negative percentage represents peak suppression, and a positive
194 percentage represents peak enhancement.

195 Dilution integrity was estimated in samples exhibiting concentrations higher
196 than the upper limit of the calibration curve which needed to be diluted. The study was
197 carried out by analyzing samples at concentrations of 15 and 75 ng/mL. The 15 ng/mL
198 sample was diluted 1:5 and the 75 ng/mL sample were diluted 1:20 before extraction.

199 Both dilution samples were analyzed in triplicate over five different runs. Stability of
200 the extracted samples placed on the rack of the instrument autosampler at 20 °C was
201 evaluated at low and high concentrations for 24 h and 48 h.

202

203 Results and discussion

204 Optimization of the extraction conditions

205 The first step of the optimizations included a fractional factorial design (2^{6-1}) using the
206 geometric means of the chromatographic peak areas of the analytes as response. The
207 result of this optimization is shown in a Pareto chart of Figure 1. Only the variables
208 sample volume and volume of SHS:HCl 6 mol/L exhibited significant influence on the
209 extraction performance. Thus, a more comprehensive optimization of these factors was
210 necessary. DMCHA was chosen as extraction solvent for further experiments because
211 presented experimental more effective phase separation step when compared to DPA.

212 The variables sample volume and volume of SHS:HCl were carefully optimized
213 through a Doehlert design. According to Figure 2, 500 µL of sample and 650 µL of
214 DMCHA:HCl 6 mol/L provided the best chromatographic responses.

215 Regarding the extraction procedure, 500 µL of whole blood and 20 µL of
216 MDMA-d_5 (IS) at concentration of 125 ng/mL were added in a 2 mL polypropylene
217 tube. Then, 250 µL of $ZnSO_4.7H_2O$ 10% and 50 µL NaOH 1 mol/L were added for
218 protein precipitation. Afterwards, the samples were vortexed for 30 s and centrifuged at
219 10,000 rpm for 5 min. The supernatant was transferred to a new polypropylene tube,
220 and 650 µL of SHS:HCl 6 mol/L mixture (1:1, v/v) was added; following the tube was

221 vortexed for 5 s and a homogeneous solution was observed. Then, 500 µL of a 10 mol/L
222 NaOH solution was added in order to promote the phase separation, the samples were
223 vortexed for 5 s and a cloudy solution was observed. The mixture was settled for 1 min
224 for phase separation, and then it was centrifuged at 10,000 rpm for 5 min. A biphasic
225 solution was observed and the organic phase containing the analytes was transferred to a
226 2 mL polypropylene tube, evaporated to dryness under N_2 flow at 40 ºC and
227 reconstituted in 30 µL of acetonitrile:H_2O mixture (5:95, v/v) with 0,1% formic acid.
228 The extract was transferred to a vial and 5 µL was injected in the LC–MS/MS.

229

230 **Method Validation**

231 After the multivariate optimizations, the experimental conditions were established, and
232 the validation was performed according to guideline of SWGTOX. The analytical
233 figures of merit obtained under the optimized conditions are shown in Table II.

234 The selectivity study was performed under the specified test conditions and no
235 significant level of interfering exogenous substances at the retention time of the analyte
236 was observed, and no carryover was observed in six chromatograms obtained after the
237 injection of an extract of the highest concentration of analytes.

238 The coefficients of determination (r^2) of the calibration curves were higher than
239 0.99. LOD and LOQ varied from 0.1 to 1.0 ng/mL. The method was linear in the studied
240 range for all analytes, with $1/x^2$ weighted linear regression for MDMA, MDA and 25C-
241 NBOMe; the calibration curves were homoscedastic for 25B-NBOMe and 25I-NBOMe.

242 Moreover, the results obtained regarding precision (within-run and between-run), biases
243 and the matrix effect are shown in Table III.

244 The within-run precision ranged from 2.16% to 9.19% and the between-run
245 precision from 2.39% to 9.59%. The bias ranged from 93.57% to 115.71%. Therefore,
246 the precision and bias results (%) in 5 days were satisfactory for all three
247 concentrations, with values lower than 20% RSD for precision study and ± 20.0%.
248 Figure 3 shows a MRM chromatogram obtained by LC–MS/MS from postmortem
249 blood samples spiked with a mixture of the analytes (3 ng/mL) and the internal standard
250 (5 ng/mL), which has been subjected to an extraction using the method developed.

251 Matrix effects evaluation ranged from 28.94 to 33.57% for MDA and 43.59 to
252 51.54% for other analytes. The ionization suppression can be occurred due to
253 components of the complex biological matrix that were not totally removed in the
254 sample preparation step. The protein precipitation with zinc sulfate can also contribute
255 with the suppression of the signal by partial binding of zinc sulfate with blood proteins
256 (37). Even with the ionization suppression, the proposed method exhibited adequate
257 sensitivity for the determination analytes in whole blood. In addition, dilution integrity
258 was also evaluated, and the results are shown in Table IV.

259 The precision and accuracy biases results (%) in 5 days were acceptable for both
260 evaluated concentrations in the dilution integrity study, 15 ng/mL (ratio 1:5) and 75
261 ng/mL (ratio 1:20), with RSD lower than 20% for precision and ± 20.0%. Within-run
262 precision ranged from 2.40% to 7.41% and between-run precision from 3.42% to
263 7.54%. The biases ranged from 102.94% to 116.50%.

264 Related to the stability of the extracts (Table V) maintained in the rack of the
265 auto-sampler (at 20 ºC), MDA and MDMA were considered stable after 48 h with
266 results ranged from 90.40 to 112.80% of the initial chromatographic peak areas. The
267 analytes 25C-NBOMe, 25B-NBOMe and 25I-NBOMe were stable for 24 h at these
268 conditions, with results ranged from 85.30 to 92.75% of the initial chromatographic
269 peak area. NBOMes presented a decreased in the percentage of the initial area at 48
270 hours higher than 20% for high concentration. Similar results were found in a previous
271 study conducted by Johnson et al. (38), showing a time-dependent stability for these
272 compounds.

273

274 **Application of the method to real samples**

275 After the optimization and validation, the proposed method was applied to four
276 postmortem whole blood samples obtained from individuals suspected to have
277 consumed psychoactive substances. MDMA and MDA were quantified in all samples
278 and the concentrations ranged from 6.91 to 68.20 ng/mL for MDMA and from 31.76 to
279 61.60 ng/mL for MDA. All samples exhibited negative results for 25C-NBOMe, 25B-
280 NBOMe and 25I-NBOMe (< LOD).

281 Our results were compared with other methods reported in the literature used for
282 the determination of these compounds in whole blood (Table VI). The proposed method
283 exhibited similar LOQ for 25B-NBOMe, 25I-NBOMe and 25C-NBOMe and lower for
284 MDA and MDMA. The sample volume used in the method was 500 µL and the sample
285 volume employed in other methods usually ranged from 100 to 1000 µL. Only the
286 method based on dried blood spot employed lower sample volume than other methods

287 (15 μL). Sample preparation time is similar to the major of other methods and suitable
288 for application in a laboratory routine. The use of organic solvents is reduced when
289 compared to traditional sample preparation techniques such LLE and SPE. Moreover,
290 few methods using microextraction for determination of these analytes appeared in the
291 literature.

292

293 **Conclusions**

294 To our knowledge, this is the first demonstration of the application of switchable
295 hydrophilic solvents as alternative extraction phases for the determination of MDA,
296 MDMA and NBOMes (25C, 25B e 25I) in postmortem blood samples. The
297 methodology was fully optimized, validated and the main advantages over traditional
298 sample preparation consisted of employing solvents with lower toxicity compared to
299 organic solvents generally used for the extraction step. Moreover, rapid extractions were
300 achieved in homogeneous phase and satisfactory LOD and LOQ were achieved using
301 LC–MS/MS. In addition, the developed method was successfully applied to identify and
302 quantify some of these analytes in human postmortem blood samples obtained from
303 individuals suspected of intoxication. SHS can be further exploited in several forensic
304 determinations since they exhibit interesting physicochemical properties allowing for
305 extractions in homogeneous media. The use of microextraction techniques and
306 alternative solvents are gaining attention in forensic analysis; however, more efforts
307 need to conducted to expand the applicability of these techniques.

308

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313

314 **Conflict of Interest**

315 There are no financial or other relations that could lead to a conflict of interest.

316

317 **References**

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487 **Figures Caption**

488 **Figure 1.** Pareto chart for the optimization of the extraction conditions.

489 **Figure 2.** Response surface for the optimization of volume of SHS:HCl 6 mol/L and
490 sample volume.

491 **Figure 3.** Multiple reaction monitoring (MRM) chromatogram obtained of postmortem
492 blood spiked with a mixture of the analytes and internal standard: (A) MDA, 3.0 ng/mL;
493 (B) MDMA-d_5, 5 ng/mL; (C) MDMA, 3.0 ng/mL; (D) 25C-NBOME, 3.0 ng/mL; (E)
494 25B-NBOMe, 3.0 ng/mL; and (F) 25I-NBOMe, 3.0 ng/mL.

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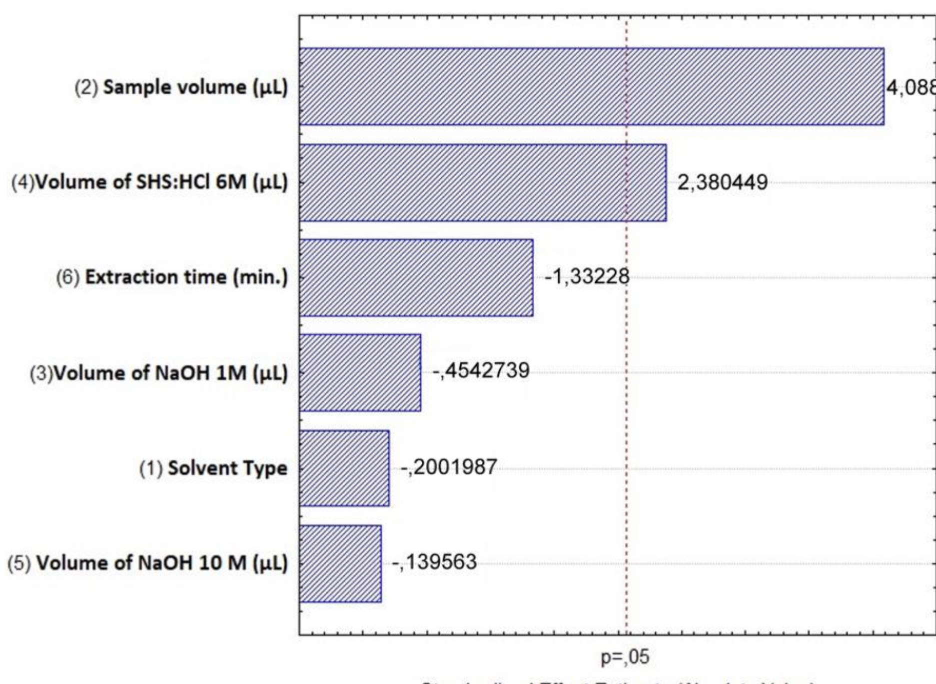
508 **Figure 1.**



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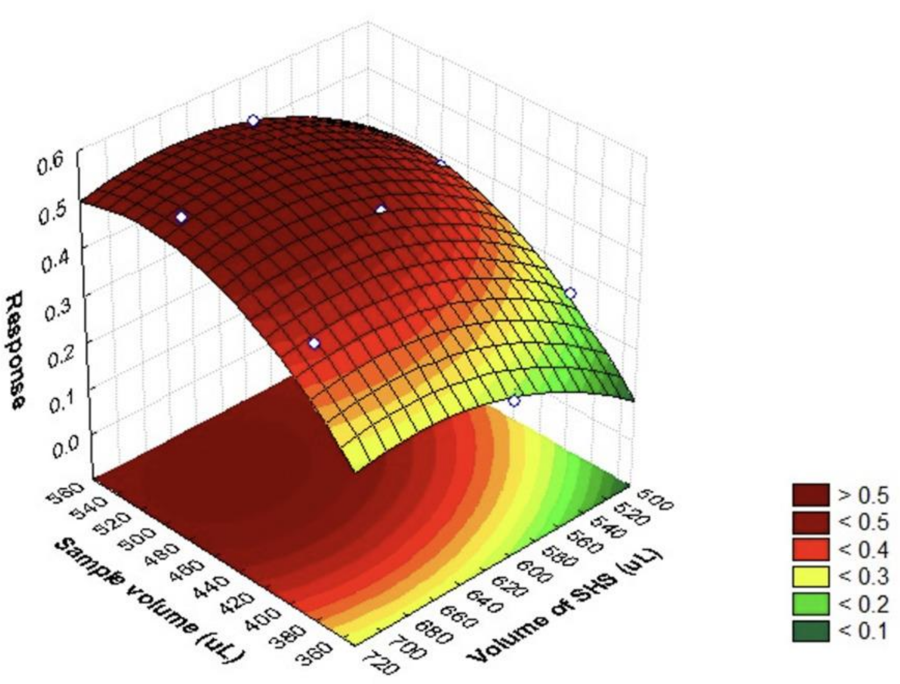
521 **Figure 2.**



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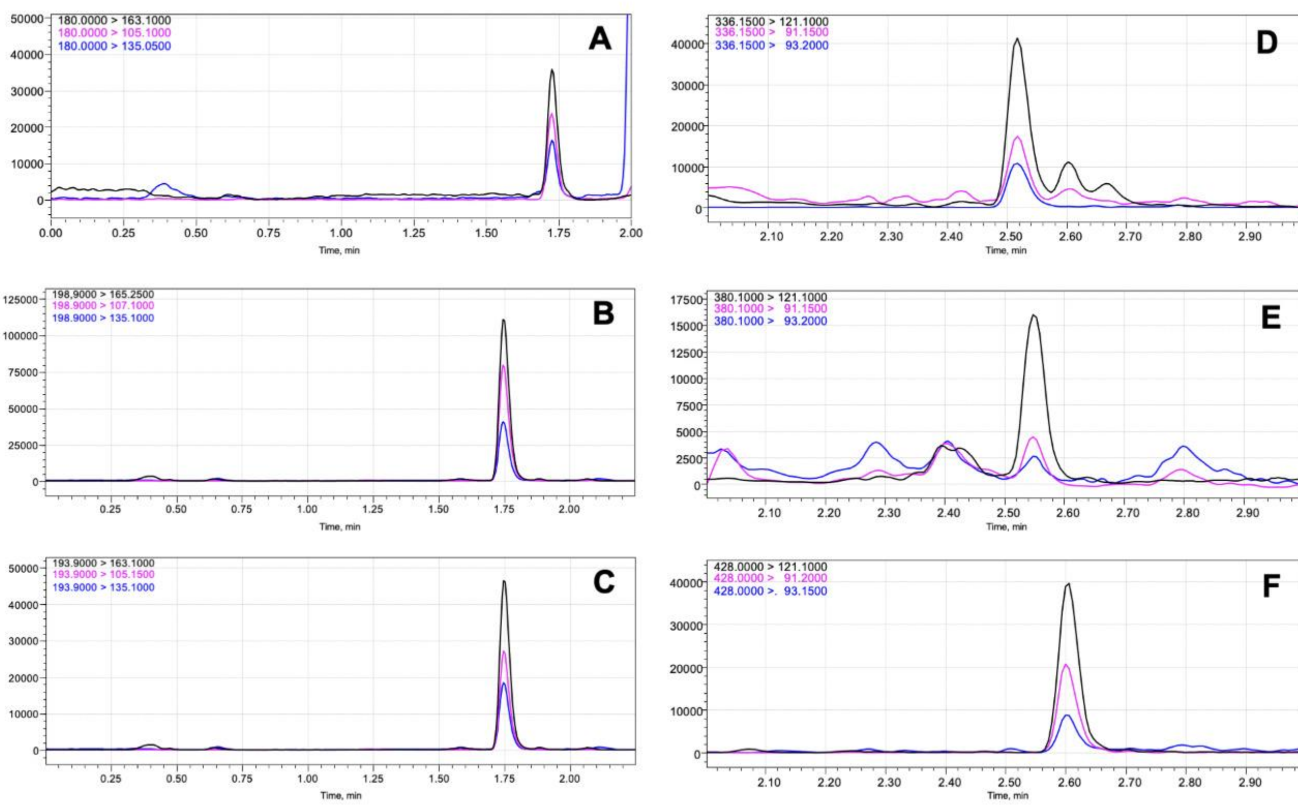
534 **Figure 3.**



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536 **Tables**

537 **Table I.** Instrumental parameters adopted in this study.

Analyte	MRM Transitions (*m/z*)	CE (eV)	Retention Time (min)
MDMA-d_5 (IS)	198.90 →165.25 (underlined) 198.90 →107.10 198.90 →135.10	-13 -25 -20	1.730
MDA	180.00 →163.10 (underlined) 180.00 → 105.10 180.00 → 135.05	-13 -22 -20	1.657
MDMA	193.90 →163.10 (underlined) 193.90 →105.15 193.90 →135.10	-12 -25 -19	1.730
25C-NBOMe	336.15 → 121.10 (underlined) 336.15 → 91.10 336.15 → 93.20	-20 -47 -39	2.499
25B-NBOMe	380.10 → 121.10 (underlined) 380.10 → 91.15 380.10 → 93.20	-21 -49 -31	2.525
25I-NBOMe	428.00 → 121.10 (underlined) 428.00 → 91.20 428.00 → 93.15	-21 -54 -46	2.587

538 Quantifier transitions were underlined.

539 MRM, multiple reaction monitoring; CE, collision energy, IS, internal standard.

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546 **Table II.** Analytical figures of merit obtained using the proposed methodology.

Analyte	Linear Equation	LOD (ng/mL)	LOQ (ng/mL)	Linear Range (ng/mL)	r^2	Weighting factor
MDA	$y = 0.0838x + 0.0119$	0.5	0.5	0.5 – 6.0	0.9906	$1/x^2$
MDMA	$y = 1.3608x + 0.0222$	0.1	0.1	0.1 – 6.0	0.9912	$1/x^2$
25C-NBOMe	$y = 0.0852x – 0.0230$	0.5	0.5	0.5 – 6.0	0.9910	$1/x^2$
25B-NBOMe	$y = 0.0580x – 0.0238$	1.0	1.0	1.0 – 6.0	0.9916	homoscedastic
25I-NBOMe	$y = 0.1218x – 0.0296$	0.5	0.5	0.5 – 6.0	0.9977	homoscedastic

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553 **Table III.** Precision, bias and matrix effect of the proposed methodology.

Analyte	Within-run Precision (%, n = 3)			Between-run Precision (%, n = 15)			Bias (%, n = 15)			Matrix Effect (%, n = 10)	
	Low[a]	**Medium[b]**	**High[c]**	**Low[a]**	**Medium[b]**	**High[c]**	**Low[a]**	**Medium[b]**	**High[c]**	**Low[a]**	**High[c]**
MDA	6.57	7.32	4.48	7.37	9.59	8.59	102.63	93.57	109.46	-33.57	-28.94
MDMA	2.41	2.92	2.16	2.74	2.75	2.39	99.34	111.69	107.12	-44.86	-50.63
25C-NBOMe	5.78	9.19	5.19	5.15	8.24	5.13	113.20	101.56	103.46	-43.59	-47.78
25B-NBOMe	5.03	6.46	5.60	5.95	5.92	5.57	115.21	110.82	109.13	-45.51	-51.54
25I-NBOMe	7.73	8.84	6.84	8.54	8.61	6.84	110.77	105.67	115.71	-45.23	-47.71

554 [a]0.3 ng/mL for MDMA; 1.5 ng/mL for MDA, 25C-NBOMe, 25I-NBOMe and 25B-NBOMe.
555 [b]2.5 ng/mL for MDMA; 3 ng/mL for MDA, 25C-NBOMe, 25I-NBOMe and 25B-NBOMe.
556 [c]4.5 ng/mL for all analytes.
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561 **Table IV.** Evaluation of the dilution integrity.

Analyte	15 ng/mL (1:5 dilution)			75 ng/mL (1:20 dilution)		
	Within-run Precision (%, n = 3)	Between-run Precision (%, n = 15)	Bias (%, n = 15)	Within-run Precision (%, n = 3)	Between-run Precision (%, n = 15)	Bias (%, n = 15)
MDA	6.67	7.54	113.75	3.34	5.07	110.62
MDMA	2.68	3.42	115.72	2.40	3.91	103.74
25C-NBOMe	5.80	5.52	114.46	6.74	6.07	102.94
25B-NBOMe	3.40	6.39	112.52	6.31	7.30	107.36
25I-NBOMe	3.89	4.50	116.50	7.41	6.49	104.29

562 **Table V.** Stability evaluation at low and high concentrations.

Analyte	Time (h)	% of chromatographic peak area	
		Low[a]	High[b]
MDA	0	100.00	100.00
	24	95.85	97.24
	48	90.40	105.08
MDMA	0	100.00	100.00
	24	108.15	112.80
	48	103.28	111.47
25C-NBOMe	0	100.00	100.00
	24	85.31	87.75
	48	77.30	69.48
25B-NBOMe	0	100.00	100.00
	24	92.75	87.12
	48	89.78	69.85
25I-NBOMe	0	100.00	100.00
	24	92.05	85.30
	48	90.52	74.79

563 [a]0.3 ng/mL for MDMA; 1.5 ng/mL for MDA, 25C-NBOMe, 25I-NBOMe and 25B-
564 NBOMe.

565 [b]4.5 ng/mL for all analytes.

566 **Table VI.** Comparison of the analytical features obtained with this study and other reported methods for the determination of substituted
567 phenethylamines in blood.
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Analyte	LOQ (ng/mL)	Sample preparation technique	Separation/ detection technique	Sample volume (µL)	Extraction solvent Volume (µL)	Sample preparation time (min)	Ref.
MDA MDMA 25C-NBOMe 25B-NBOMe 25I-NBOMe	0.5 0.1 0.5 1.0 0.5	SHS-HLLME	LC–MS/MS	500	650 µL SHS	> 11[a]	This study
25B-NBOMe	0.0159	LLE	LC–MS/MS	200	2 mL ethyl acetate	> 6[a]	(39)
25I-NBOMe	0.1	PP	LC–MS/MS	200	600 µL acetonitrile	> 10[a]	(40)
25C-NBOMe 25B-NBOMe 25I-NBOMe	0.5	SPE and derivatization	GC–MS	1000	4 mL methanol + 3 mL dichloromethane/isopropranol/ammonia (78:20:2, v/v)[b]	> 11[a,c]	(41)
25B-NBOMe 25C-NBOMe 25I-NBOMe	0.1	DBS	LC–MS/MS	15	IS solution - 300 µL methanol/acetonitrile (75:25, v/v)	195	(19)

25B-NBOMe 25C-NBOMe 25I-NBOMe	0.01 0.02 0.01	SPE	LC–MS/MS	500	3 mL methanol + 3 mL of 2% ammonium hydroxide in methylene chloride:isopropanol (80:20)[b]	> 4[a,b,c]	(38)
25B-NBOMe	1.0	LLE	LC–MS	100	2 mL hexane/2-propanol (9:1, v/v)	> 15[a]	(42)
25B-NBOMe 25C-NBOMe 25I-NBOMe	0.1	LLE	LC–MS/MS	300	1.2 mL MTBE	> 10[a]	(43)
25I-NBOMe	0.025	SPE	LC–MS/MS	1000	6 mL methanol + 3 mL dichloromethane/isopropranol/ammonia (78:20:2)[b]	> 9[a,b,c]	(15)
25B-NBOMe 25I-NBOMe	0.5	PP	LC–MS/MS	250	1 mL acetonitrile (containing PI)	> 5[a]	(13)
25B-NBOMe 25C-NBOMe 25I-NBOMe	0.08 0.08 0.10	SPE	LC–MS/MS	1000	7 mL methanol + 4 mL dichloromethane:isopropanol containing 2% ammonia (8:2)	-	(10)
25B-NBOMe 25C-NBOMe	50	LLE and sonication	UHPLC–QTOF	100	3 mL acetonitrile	> 50[a]	(44)

25I-NBOMe							
MDA MDMA 25C-NBOMe 25B-NBOMe 25I-NBOMe	-	LLE	LC–MS/MS	500	1 mL dichloromethane/butyl chloride 1:4 (v/v)	> 28[a]	(45)
MDA MDMA	5	PP	LC–MS/MS	250	1 mL acetonitrile	13[a]	(2)
MDA MDMA	0.65 – 2.4	LLE and derivatization	GC–MS/MS	200	2 mL ethyl acetate	> 28[a]	(46)
MDMA	30	dispersive-SPE	GC–MS	1000	2 mL acetonitrile	> 10	(11)

SHS-HLLME, switchable hydrophilicity solvent-based homogenous liquid–liquid microextraction; LLE, liquid-liquid extraction; PP, protein precipitation; SPE, solid-phase extraction.

[a] the evaporation time was not considered in the extraction time.

[b] volume extraction = organic solvent used for conditioned of the SPE cartridges, clean up and elution.

[c] the time of the SPE extraction step is not considered

4. CONCLUSÕES

Neste trabalho, pela primeira vez, solventes de hidrofilicidade comutável foram usados como fase de extração alternativa para a determinação das drogas sintéticas MDA, MDMA e NBOMes (25B, 25C e 25I) em amostras de sangue *postmortem*. A metodologia foi totalmente otimizada e validada conforme recomendações internacionais, confirmando a sua aplicabilidade nas análises toxicológicas forenses. Além disso, os limites de detecção e quantificação obtidos foram satisfatórios, considerando as concentrações destes analitos em casos de intoxicação fatal já reportadas na literatura. As principais vantagens da técnica de preparo de amostras proposta com relação às técnicas de preparo de amostras clássicas (LLE e SPE) está no emprego de menores volumes de solventes e solventes menos tóxicos, apresentando concordância com os preceitos da química analítica verde.

O método desenvolvido foi aplicado com sucesso em amostras de sangue total humano obtidas de indivíduos com suspeita de intoxicação, sendo identificados e quantificados os analitos MDA e MDMA em todas as amostras fornecidas pelo Departamento de Perícias Laboratoriais do Instituto Geral de Perícias (DPL-IGP) do Rio Grande do Sul.

Diante do exposto, esse trabalho destaca-se por contribuições nas áreas da toxicologia analítica e química verde, através do desenvolvimento e validação de um método analítico para aplicação na área forense e pela utilização do solvente de hidrofilicidade comutável, que possibilitou o desenvolvimento de um método mais ambientalmente amigável. Além disso, os SHSs possuem potencial para serem explorados em uma série de determinações forenses, uma vez que apresentam propriedades físico-químicas interessantes, permitindo extrações rápidas e eficientes em meios homogêneos.

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6. ANEXOS

6.1 ANEXO I – PARECER DO COMITÊ DE ÉTICA EM PESQUISA

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PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Desenvolvimento de métodos analíticos para identificação de novas substâncias psicoativas de interesse forense por espectrometria de massas de alta resolução

Pesquisador: TIAGO FRANCO DE OLIVEIRA
Área Temática:
Versão: 2
CAAE: 17996819.7.0000.5345
Instituição Proponente: Universidade Federal de Ciências da Saúde de Porto Alegre
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.784.184

Apresentação do Projeto:
As drogas naturais, como maconha e cocaína, foram gradativamente substituídas pelas sintéticas. O recente relatório intitulado "Global Synthetic Drugs Assessment", publicado pelo United Nations Office on Drugs and Crime - UNODC, revelou que a produção e o consumo de drogas sintéticas têm alcançado números alarmantes, superando os da heroína e
cocaína em muitos lugares do mundo. Segundo o relatório global, foram registradas 348 Novas Substâncias Psicoativas (NPS), de 2008 a 2013, mas o número real de NPS disponível no mundo pode ser significativamente superior, dado que esses números refletem apenas relatos de fontes oficiais e não leva em conta fontes não oficiais. As assim chamadas drogas sintéticas são substâncias ou misturas de substâncias psicoativas produzidas por síntese química a partir de substâncias precursoras encontradas ou não na natureza. A dimensão e os padrões de uso dessas substâncias ainda não são claros e, provavelmente, estão sendo subestimados. Diante do exposto, o objetivo desse projeto é o desenvolvimento de metodologias analíticas para a correta identificação de NPSs (etilona, 2,5-dimetoxi-4-bromoanfetamina, 2,5-dimetoxi-4-metilanfetamina, 25CNBOMe, 25B-NBOMe) e outras substâncias psicoativas em matrizes biológicas por espectrometria de massas de alta resolução. As metodologias aqui desenvolvidas serão disponibilizadas para o Departamento de Perícias Laboratoriais do Instituto Geral de Perícias (DPL-IGP) para a posterior

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implementação na rotina laboratorial, objetivando a elucidação e o mapeamento de casos de envolvendo o consumo de NPSs e outras substâncias psicoativas.

Objetivo da Pesquisa:

1) Objetivo Primário:

Desenvolvimento de metodologias analíticas para a correta identificação de NPSs e substâncias psicoativas convencionais em matrizes biológicas por espectrometria de massas de alta resolução, fornecendo assim subsídios para elucidação de casos com suspeita de ocorrência dessa classe de substâncias.

2)Objetivo Secundário:

a)desenvolvimento de métodos multi-analiticos que permitam a análise simultânea em um intervalo curto de tempo, propiciando uma identificação rápida e precisa das substâncias envolvidas em casos suspeitos de NPS;

b)desenvolvimento e validação de estratégias analíticas de screening em cabelo para as classes de substâncias anfetaminas, antidepressivos, antipsicóticos, barbitúricos, benzodiazepínicos e cocaína;

c)desenvolvimento tecnológico adquirido poderá ser facilmente aplicado a outros projetos de pesquisa, contribuindo assim para avanços importantes no que concerne às implicações da toxicologia analítica à ciências forenses;

d)após a validação metodológica, os procedimentos desenvolvidos estarão acessíveis para Departamento de Perícias Laboratoriais do Instituto Geral de Perícias (DPLIGP);

e) para realizar a transferência do know-how as metodologias serão prioritariamente construídas considerando os métodos de rotina e o parque instrumental do DPL-IGP;

f) os dados gerados no trabalho serão tabulados para confecção de artigos científicos construídos em parceria com a equipe do DPL-IGP.

Avaliação dos Riscos e Benefícios:

Riscos:

Os pesquisadores descrevem que não há riscos para os envolvidos na pesquisa pois as amostras biológicas utilizadas serão oriundas do descarte do laboratório do DPL-IGP. As
amostras terão seu uso liberado pelo responsável do laboratório somente após estarem em posição de descarte. Referem ainda que o possível risco é referente a identificação dos indivíduos, no entanto, os pesquisadores garantem que a identificação dos indivíduos será mantida em anonimato, através da utilização de códigos previamente estabelecidos.

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Benefícios:
Não haverá benefícios diretos ao participante da pesquisa, mas o estudo será importante pois será desenvolvido uma metodologia capaz de identificar diferentes substâncias psicoativas em diversas matrizes biológicas que possa ser disponibilizada para os principais serviços de avaliação de intoxicações por drogas de abuso no estado do Rio Grande do Sul. Além disto, espera-se a identificação destes compostos em casos suspeitos possibilitando o mapeamento da distribuição das substâncias elencadas.

Comentários e Considerações sobre a Pesquisa:
Amostra: amostras biológicas de rotina (sangue, urina, vísceras) ou de oportunidade (humor vítreo e cabelo) disponibilizadas pelo DPL-IGP de casos com suspeita de ocorrência de NPS, passíveis de análises toxicológicas, atendidos pelo Instituto durante o período de vigência do projeto. Atualmente, estas amostras são analisadas na rotina Departamento de Perícias Laboratoriais e descartadas, conforme a legislação vigente (ANVISA, RDC 306/04, CONAMA, RDC 358/05), que por tratar de resíduos do Grupo A1 são submetidos a processos de tratamento em equipamentos que promova a redução de carga microbiana e encaminhados para aterro sanitário licenciado para disposição final destes resíduos.

Considerações sobre os Termos de apresentação obrigatória:
- Solicitam a utilização apenas do TCUD, tendo em vista a impossibilidade de obtenção do TCLE, uma vez que se trata de amostras biológicas oriundas do descarte do laboratório do DPL-IGP e que terão seu uso liberado pelo responsável do laboratório somente após estarem em posição de descarte.
- Não encontra-se anexado o termo de compromisso para entrega dos relatórios parciais e final.

Recomendações:
- O projeto somente poderá ter início após sua aprovação na integralidade pelos CEP's envolvidos.
- Solicita-se encaminhar por notificação o termo de compromisso para entrega dos relatórios parciais e final. Como trata-se e um projeto com período de realização abrangente, os relatórios parciais devem ser anuais além do relatório final. Destaca-se a importância da entrega destes relatórios para acompanhamento do CEP, além de possibilitar ao pesquisador, dentro da vigência do mesmo, o envio de qualquer emenda/notificação.
- Data Final de Vigência do Projeto: 01/09/2023.

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Conclusões ou Pendências e Lista de Inadequações:

- Os pesquisadores atenderam às solicitações contidas em parecer emitido anteriormente por este CEP.

Considerações Finais a critério do CEP:

De acordo com o parecer do Relator.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BASICAS_DO_PROJETO_1401883.pdf	06/11/2019 15:31:08		Aceito
Outros	TCUD.pdf	06/11/2019 15:30:43	TIAGO FRANCO DE OLIVEIRA	Aceito
Declaração de Instituição e Infraestrutura	TemoAnuenciaGerLab.pdf	06/11/2019 15:30:30	TIAGO FRANCO DE OLIVEIRA	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_parceria_UFCSPA_IGP.docx	23/07/2019 16:09:50	TIAGO FRANCO DE OLIVEIRA	Aceito
Outros	Termo_liberacao_amostras.pdf	23/07/2019 16:09:32	TIAGO FRANCO DE OLIVEIRA	Aceito
Outros	Termo_anuencia_instituicao_coparticipante.pdf	23/07/2019 16:09:11	TIAGO FRANCO DE OLIVEIRA	Aceito
Folha de Rosto	folhaDeRosto_Tiago_Oliveira.pdf	23/07/2019 16:06:23	TIAGO FRANCO DE OLIVEIRA	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

PORTO ALEGRE, 19 de Dezembro de 2019

Assinado por:
Luciane Dalcanale Moussalle
(Coordenador(a))

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6.2 ANEXO II – NORMAS DE PUBLICAÇÃO DA REVISTA

Instructions to Authors

Scope

Ethics

Peer Review Process

Conflict of Interest

Experimental Subjects

Manuscript Submission

Manuscript Preparation

Submitting a Manuscript

Preparing Documents for Submission

Safety Considerations

Figure/Table Permissions

Figure Submission

Color Figures

Supplementary Data

Analytical Method Validation

References

Proofs

License to Publish

Open Access Option for Authors

Funding

About Advance Access Publication

Author Self-Archiving/Public Access policy

Communications

Scope

JAT accepts for consideration articles relating to the isolation, identification, quantitation, and interpretation of potentially toxic substances and their biotransformation products in specimens of human and animal origin. The articles should pertain especially to the monitoring of drugs and therapeutic agents and environmental and industrial contaminants, clinical reports of poisonings (with analytical data), the development of analytical techniques, and the interpretation of the results of toxicological investigations. The methods should be applicable to the fields of forensic science, therapeutic drug monitoring, drugs-of-abuse testing, clinical and forensic toxicology, and industrial hygiene. The manuscript should conform to one of the following types: Article, Book Review, Case Report, Technical Note, Review or Letter to Editor.

Ethics

Submission of a paper to JAT implies that the manuscript has not been published in and is not under concurrent consideration by any other journal and that the author(s) have obtained appropriate permission to use data obtained for and contained in the manuscript. Previous presentation at professional meetings should be mentioned in a footnote.

Plagiarism will result in the immediate rejection of a manuscript, and the involved authors will be banned from future publication in JAT. Any manuscript submitted to JAT is subject to review by iThenticate software to assist in the detection of plagiarism and may be rejected as the result of this review.

Additional information about publication ethics policies can be found on our webpage.

Peer Review Process

All manuscripts submitted to the Journal are reviewed by the Editor. Those outside the scope of the Journal are rejected without peer review; all other manuscripts are sent to independent experts for anonymous peer review. The reviewers make an independent scientific assessment and a recommendation to the Editor. The Editor considers the manuscript and the reviewers' comments before making the decision

to accept, accept pending revision, or reject a manuscript. Acceptance pending revision does not guarantee eventual acceptance.

The average time from submission to first decision is four weeks.

Revisions

Manuscripts accepted pending revision must be returned to the editorial office within 30 or 60 days of the date of the decision letter for minor or major revision, respectively. If an extension is necessary, contact the editorial office. Authors should include a point-by-point response to the reviewers' and Editor's comments and indicate where changes made based on these comments can be found in the revised manuscript. All revisions to the text must be clearly indicated, such as by track changes, differently colored text and/or highlighting.

Any alteration in authorship, including a change in order of authors, must be agreed upon by all authors, and a statement signed by all authors must be submitted to the editorial office.

Before making a final decision on publication status, the Editor reviews all revised manuscripts to ensure the authors fully and properly address the reviewers' concerns and comments and the revised manuscript meets the Journal's standards of scientific merit, relevance, and novelty. Revised manuscripts are subject to further review at the Editor's discretion.

Appeals

Authors may appeal the Editor's decision if they think it is unwarranted. Appeals must be submitted by email to the editorial office and include explicit detail as to why the manuscript should be reconsidered. Please do not resubmit your article without contacting the editorial office first.

Conflicts of Interest

All potential conflicts of interest must be disclosed at submission. Each author must complete the ICMJE form, and the forms are to be submitted along with the manuscript files. More information can be found here.

Experimental Subjects

When human subjects are used, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject. Authors should be aware of the Code of Ethics of the World Medical Association (Declaration of Helsinki), which has been printed in the *British Medical Journal* (July 18, 1964). When experimental animals are used, the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the guidelines published in the *NIH Guide for the Care and Use of Laboratory Animals* (NIH publication no. 86-23, revised 1987) or the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Manuscript Submission

Submission of a paper to this journal implies that the manuscript has not been published in, or submitted to, any other journal and that the author(s) have obtained appropriate permission to use data obtained for and contained in the manuscript. Previous presentation at professional meetings should be mentioned in a footnote. Manuscripts should be in their final form when they are submitted, so that proofs require only correction of typographical errors. All parts of the manuscript (except figures) should be double-spaced throughout and should be in a word-processing format (e.g., .docx, .doc, .rtf, etc.).

Manuscript Preparation

The following table should be used to choose the type of manuscript to be submitted. It may be appropriate to submit additional information as supplemental data, which will not count towards the word limits described.*

*Letters to the editor, book reviews, errata and corrigenda are published on-line only.

Manuscript Type	Description	Length

Article	An original research article reports a study within the scope of the Journal. The article must be a full scientific paper. It should present research results of scientific merit, novelty, and significance.	Up to 7000 words, including the Abstract, References (~1000 words), and Tables and Figures (considered 500 words each).
Technical Note	A Technical Note describes the process, progress, or results of technical or scientific research.	Up to 4000 words, including the Abstract, References (~1000 words), and Tables and Figures (considered 500 words each).
Book Review	A Book Review is typically an invited review of a book that is relevant to the Journal's scope.	Up to approximately 1000 words; it should be written as concisely as possible.
Case Report	A Case Report presents the details of real cases from practice within the scope of the Journal. If results are presented, the report should provide sufficient validation details such that the results presented can be evaluated for quality.	Up to 4000 words, including the Abstract, References (~1000 words), and Tables and Figures (considered 500 words each); it should contain a case history.
Review	A Review contains a comprehensive survey of topic(s) within the Journal's scope. It should provide a critique of the current literature. It may be invited or interested authors should send an email to the Editor prior to submission.	Up to 10000 words, including the Abstract, References (~1000 words), and Tables and Figures (considered 500 words each).
Letter to the Editor	A Letter to the Editor is generally about the Journal's prior publication or a communication about a policy that would fall within the Journal's scope.	Up to 1000 words, including References (~1000 words), and Tables and Figures (considered 500 words each).

Manuscripts should be subdivided into the following sequence of sections, as applicable:

- Title page
- Abstract
- Keywords
- Introduction

- Experimental
- Materials
- Methods
- Results
- Discussion
- Conclusion
- Funding
- Acknowledgements
- References
- Tables
- Legends to figures
- Figures (if not in a graphic-type file like PDF, tif, eps, etc.)
- Supplementary data

The text should describe the equipment and method(s) in sufficient detail to permit replication of the results. Further details regarding validation requirements are found below in "Analytical Method Validation."

JAT is an English-language journal. Manuscripts should be written clearly and concisely according to the rules of standard English grammar, and the Editors reserve the right to reject poorly written papers without review. Authors whose first language is not English are encouraged to have their manuscripts reviewed by an editor whose first language is English.

One to ten keywords can be entered at the time of submission.

The Abstract should be no longer than 300 words, be comprehensible to readers, and not contain reference citations.

Case Report manuscripts should not exceed 4000 words, including the Abstract, References (~1000 words), and Tables and Figures (considered 500 words each). Case Reports should include a case history.

Tables must be separate from the manuscript text, numbered consecutively with Roman numerals, cited in the text consecutively, and include a brief descriptive title. Footnotes are acceptable but should not include extensive experimental information.

Any third-party material included must have permission to reproduce from the publisher/rights holder already secured and be properly credited within the manuscript. Documentation should be submitted along with the manuscript. (Additional information may be found here.)

Availability of Data and Materials

Where ethically feasible, JAT strongly encourages authors to make all data and software code on which the conclusions of the paper rely available to readers. Authors are required to include a Data Availability Statement in their article.

We suggest that data be presented in the main manuscript or additional supporting files, or deposited in a public repository whenever possible. For information on general repositories for all data types, and a list of recommended repositories by subject area, please see Choosing where to archive your data.

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The inclusion of a Data Availability Statement is a requirement for articles published in *JAT*. Data Availability Statements provide a standardised format for readers to understand the availability of data underlying the research results described in the article. The statement may refer to original data generated in the course of the study or to third-party data analysed in the article. The statement should describe and provide means of access, where possible, by linking to the data or providing the required unique identifier.

The Data Availability Statement should be included in the endmatter of your article under the heading 'Data availability'.

More information and examples of Data Availability Statements.

Data Citation

JAT supports the Force 11 Data Citation Principles and requires that all publicly available datasets be fully referenced in the reference list with an accession number or unique identifier such as a digital object identifier (DOI). Data citations should include the minimum information recommended by DataCite:

- [dataset]* Authors, Year, Title, Publisher (repository or archive name), Identifier

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Figure Submission

1. Figure Types

Line Art
Line art has sharp, clean lines and geometrical shapes against a white background. Line art is typically used for tables, charts, graphs, and gene sequences. You can use a program like Illustrator to create high-quality line art. A minimum resolution of 300 ppi will maintain the crisp edges of the lines and shapes.

- Format: TIFF or EPS
- Minimum Resolution: 300 ppi

Grayscale
Grayscale figures contain varying tones of black and white. They contain no color, so grayscale is synonymous with "black and white." The gray scale is divided into 256 sections with black at 0 and white at 255. Software for preparation of grayscale art includes Photoshop.

- Format: TIFF or EPS
- Minimum Resolution: 300 ppi

Halftones
The best example of a halftone is a photograph, but halftones include any image that uses continuous shading or blending of colors or grays, such as gels, stains, microarrays, brain scans, and molecular structures. To prepare and manipulate halftone images, use Photoshop or a comparable photo-editing program.

- Format: TIFF
- Minimum Resolution: 300 ppi

Combination Figures
Combination figures contain two or more types of images, for example, a halftone figure containing text. You should embed the images, group the objects, or flatten the layers, and flatten transparencies before saving as TIFF at a minimum of 300 ppi.

- Format: TIFF
- Minimum Resolution: 300 ppi

Convert PowerPoint Files to High-Resolution TIFFs

Caution: Do not add artwork to your PowerPoint slides by copying from another application and then pasting into PowerPoint. Your figures will be downsampled to screen resolution. Instead use Insert > Picture > From File.

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There are two possible ways to create PDFs from PowerPoint files: use the Adobe PDF menu in some versions of PowerPoint, or create a PDF via the Print command.

1. Open your file in PowerPoint. From the Adobe PDF menu, select Change Conversion Settings. The PDFMaker Settings dialog displays.
2. From the Conversion settings dropdown menu, select Press Quality. Uncheck View Adobe PDF result. Click OK.
3. From the Adobe PDF menu, select Convert to Adobe PDF. You will be asked to save the PDF file to a location of your choosing.
4. Click OK.

– OR –

1. Open your file in PowerPoint.
2. Select Print from the File dropdown menu.
3. Select the Adobe PDF (or similar driver) in the Printer Name window.
4. Click Properties. Change the Default Settings pull-down to Press Quality. Uncheck the "View Adobe PDF results" box if you don't want Acrobat to launch.
5. Click OK, then click OK. Pick where the PDF will be created, and click Save. Note: If your PowerPoint file contains figures on multiple slides, print each slide to a separate PDF (if you do this, skip ahead to Step III). Alternately, you can create one PDF file and then use Adobe Acrobat to separate the figures/slides into individual files, as detailed in Step II.

Step II: Convert Multi-Page PDF File to Individual Files

1. Using Adobe Acrobat Standard, open the PDF file that you created in Step 1. From the Document menu, select Pages and then Extract. The Extract Page dialog box displays.

2. Enter the page numbers in the To and From fields and then select the Delete Pages checkbox. Checking this box will delete the page that you entered in the To and From fields from the PDF file.

3. Click OK. The page that you specify in the previous step is now shown in Acrobat.

4. From the File menu, select save and enter the file name (e.g., Figure 1) for the extracted page and then click OK.

5. Repeat this process until a separate file is created for each figure/slide.

Step III: Convert Individual PDF Files to TIFFs

In Photoshop:

1. File→Open the PDF. You will need to do this one page at a time. Make sure you're importing it at 300ppi, RGB.

2. Use the Crop Tool (fifth from the top of the toolbar) to select an area close to the borders of your image. Hit Enter to apply the crop.

3. Layer→Flatten Image

4. Image→Image Size. Uncheck the Resample Image checkbox. If the Width is over 17.35cm, type 17.35 in the Width box (17.35cm is our maximum allowable width for figures). The Resolution will go up automatically as the Width decreases. If the resolution does not hit 300 when you make the Width 17.35, type 300 in Resolution and as long as Width doesn't go below 8.3cm, everything is fine. Also, the height cannot be more than 23.35. If the Height and Width are within these prescribed limits, no adjustment to your figure size needs to be made.

5. File→Save As. Save as TIFF, Image Compression set to LZW, Pixel Order set to Interleaved, Byte Order set to IBM PC.

In GIMP:

1. File→Open the PDF. You will need to do this one page at a time. Open pages as Images at 300ppi. Click Import.

2. Use the Crop Tool (third row, second from the right, looks like a knife blade) to select an area close to the borders of your image. Hit Enter to apply the crop.

3. Image→Scale Image. Set the units of measurement, in the pull down menu next to Height, to millimeters. If the Width is over 173.5mm, type 173.5 in the Width box (17.35cm is our maximum allowable width for figures) and hit Tab. The new Height of the figure will appear, scaled proportionately to the change in Width. The Width cannot be below 83.0mm, and the height cannot be more than 233.5mm. If the Height and Width are within these prescribed limits, no adjustment to your figure size needs to be made.

4. File→Save As. Click the + sign next to "Select File Type (By Extension)". From the menu that appears, select TIFF. Click Save. Set Compression set to LZW. If you're prompted about layers in the file, select Flatten Image.

In Acrobat Pro:

1. File→Open the PDF

2. If necessary, go Document→Rotate Pages to rotate the document to a horizontal orientation.

3. File→Save As. In the "Save as type" pull-down menu, select TIFF.

4. Click the Settings button on the right-hand side of the Save As dialog box. In the top third, under "File Settings", both Grayscale and Color should be set to LZW. In the bottom third, "Conversion," set Colorspace to Color:RGB, and Resolution to 300ppi. Click OK. Click Save.

Note: PDFs converted to TIFFs in this manner should still be opened in Photoshop or GIMP to crop excess white space, and make sure the figure falls within our maximums and minimums.

Convert Excel or Word Files to High-Resolution TIFFs

Windows 98, XP, Vista and Excel/Word 2003 or 2007:

Step I: Convert Excel/Word File to PDF

There are two possible ways to create PDFs from Excel/Word files: use the Adobe PDF menu in some versions of Excel/Word, or create a PDF via the Print command.

1. Open your file in Excel/Word. From the Adobe PDF menu, select Change Conversion Settings. The PDFMaker Settings dialog displays.

2. From the Conversion settings dropdown menu, select Press Quality. Uncheck View Adobe PDF result. Click OK.

3. From the Adobe PDF menu, select Convert to Adobe PDF. You will be asked to save the PDF file to a location of your choosing.

4. Click OK.

– OR –

1. Open your file in Excel/Word.
2. Select Print from the File dropdown menu.
3. Select the Adobe PDF (or similar driver) in the Printer Name window.
4. Click Properties. Change the Default Settings pull-down to Press Quality. Uncheck the "View Adobe PDF results" box if you don't want Acrobat to launch.
5. Click OK, then click OK. Pick where the PDF will be created, and click Save.

Step II: Convert Individual PDF Files to TIFFs

In Photoshop:

1. File→Open the PDF. You will need to do this one page at a time. Make sure you're importing it at 300ppi, RGB.
2. Use the Crop Tool (fifth from the top of the toolbar) to select an area close to the borders of your image. Hit Enter to apply the crop.
3. Layer→Flatten Image
4. Image→Image Size. Uncheck the Resample Image checkbox. If the Width is over 17.35cm, type 17.35 in the Width box (17.35cm is our maximum allowable width for figures). The Resolution will go up automatically as the Width decreases. If the resolution does not hit 300 when you make the Width 17.35, type 300 in Resolution and as long as Width doesn't go below 8.3cm, everything is fine. Also, the height cannot be more than 23.35. If the Height and Width are within these prescribed limits, no adjustment to your figure size needs to be made.
5. File→Save As. Save as TIFF, Image Compression set to LZW, Pixel Order set to Interleaved, Byte Order set to IBM PC.

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Additional clarification on tables and figures can be found on the Author Services webpage.

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References

Journal article (already published in an issue):

Lee, D. and Stout, P. (2020) Toxicological and demographic profiles of phencyclidine-impaired driving cases in Houston. Journal of Analytical Toxicology, 44, 499–503.

Feng, S., Enders, J.R., Cummings, O.T., Strickland, E.C., McIntire, T., and McIntire, G. (2020) A dilute and shoot LC–MS-MS method for antipsychotics in urine. Journal of Analytical Toxicology, 44, 331–338.

Journal article (e-pub ahead of print):

Mancini, R., Fernandez-Lopez, L., Falcon, M., Pellegrini, M, Luna, A., and Rotolo, M. (2020) Postmortem analysis of benzodiazepines in human bone by gas chromatography–mass spectrometry. Journal of Analytical Toxicology. doi: 10.1093/jat/bkaa020

Chapter in a book:

Felsot, A., (2006) The evolution of agricultural practices, pest control technologies, and public expectations. In Felsot, A. and Racke, K. (eds.), Certified organic and biologically derived pesticides, 1st edition, Chapter 1. Oxford University Press, New York, NY, pp. 5–l30.

Jones, A.W. (2011) Driving under the influence of alcohol. In Moffat, A.C., Osselton, M.D., Widdop, B., and Watts, J. (eds.), Clarke's analysis of drugs and poisons, 4th edition, Volume 1, Chapter 4. Pharmaceutical Press, London, U.K., pp. 87–114.

Book (Editor as author):

Tu, A. and Gaffield, W. (eds.) (2000) Natural and selected synthetic toxins. Oxford University Press, New York, NY, pp. 50–105.

Book (Editor as author):

Tu, A. and Gaffield, W. (eds.) (2000) Natural and selected synthetic toxins. Oxford University Press, New York, NY, pp. 50–105.

Book (no Editor):

Baselt, R.C. (2017) Cocaine. In Disposition of toxic drugs and chemicals in man, 11th edition. Biomedical Publications, Seal Beach, CA, pp. 527–531.

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Three to six authors: Huestis, M.A., Cone, E.J. and Caplan, Y.H.
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Electronic source:

(2006) The Forensic Toxicology Laboratory Guidelines—2006 Version. Society of Forensic Toxicologists/American Academy of Forensic Sciences. http://soft-tox.org/files/Guidelines_2006_Final.pdf (accessed August 14, 2017).

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