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DETERMINAÇÃO DE COMPOSTOS NÃO-ALVO POR HRMS E QUANTIFICAÇÃO DE CAFEÍNA POR RMN EM DROGAS USADAS POR MOTORISTAS PROFISSIONAIS NO BRASIL

Tainara Guizolfi

CAXIAS DO SUL
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Dissertação apresentada ao Programa de Pós-graduação em Biotecnologia da Universidade de Caxias do Sul, como parte dos requisitos para a obtenção de grau de Mestre em Biotecnologia.

Orientador: Prof. Dr. Sidnei Moura e Silva
Co-Orientador Airton Carlos Kraemer

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"Writing is magic, as much the water of life as any other creative art. The water is free. So, drink. Drink and be filled up."

Stephen King

RESUMO

As rodovias são fundamentais para a economia brasileira, pois é a principal via para o transporte de cargas, onde cerca de 75% de todas as mercadorias são transportadas. Ademais, a pressão econômica leva esses motoristas a trabalhar longas horas devido aos prazos curtos, levando a jornadas de trabalho exaustivas e a uma baixa remuneração. Com isso, há um aumento significativo no uso de estimulantes por esses profissionais, entre estes compostos podemos destacar o uso recorrente de anfetamínicos, cocaína e seus derivados. Muitas vezes esses estimulantes são comprados na forma de rebites em postos de gasolina. Desta forma, este trabalho busca identificar compostos ativos não-alvo em amostras conhecidas como "Rebites" utilizadas por motoristas profissionais de cargas pesadas no Brasil através de várias técnicas analíticas. Para isso, foram analisados sete comprimidos de rebite por espectrômetro de massa de alta resolução por infusão direta, onde é possível identificar cafeína, como composto majoritário. A partir disso, seis comprimidos independentes foram avaliados por ressonância magnética nuclear quantitativa a fim de quantificar esse composto, onde obteve-se em torno de 14% até 30% de cafeína. Ainda, as amostras foram analisadas por cromatografia a líquido para avaliar a confiabilidade da Ressonância Magnética Nuclear Quantitativa e por *Field Emission Electron Guns- Energy dispersive X-ray spectroscopy* como possível método de análise para o âmbito forense. Os compostos ativos presentes nos comprimidos testados não são detectados nos exames toxicológicos atuais. Os resultados obtidos pela cromatografia líquida necessita de ajustes no método analítico para que seus resultados sejam mais próximos dos obtidos pela técnica da Ressonância Magnética Nuclear Quantitativa, porém a técnica de ressonância pode ser utilizada na área forense como complemento à espectrometria de massas, além de demonstrar sua viabilidade diante dos resultados obtidos.

Palavras chave: Rebites; Estimulantes; Cafeína; q-RMN.

ABSTRACT

Highways are fundamental to the Brazilian economy, as it is the main route for cargo transportation, where around 75% of all goods are transported. Furthermore, economic pressure pushes these drivers to work long hours due to tight deadlines, leading to exhausting working hours and low pay. With that, there is a significant increase in the use of stimulants by these professionals, among these compounds we can highlight the recurrent use of amphetamines, cocaine and its derivatives. Often these stimulants are purchased in the form of rivets at gas stations. In this way, this work seeks to identify target and non-target active compounds in samples known as "Rebites" used by professional drivers of heavy loads in Brazil through various analytical techniques. For this, seven rivet tablets were analyzed by direct infusion high-resolution mass spectrometer, where it is possible to identify caffeine as the major compound. From this, six independent pills were evaluated by quantitative nuclear magnetic resonance in order to quantify this compound, where around 14% to 30% of caffeine was obtained. Still, the samples were analyzed by liquid chromatography to evaluate the reliability of the Quantitative Nuclear Magnetic Resonance and by Field Emission Electron Guns-Energy dispersive X-ray spectroscopy as a possible method of analysis for the forensic scope. The active compounds present in the tested pills are not detected in current toxicological tests. The results obtained by liquid chromatography require adjustments in the analytical method so that their results are closer to those obtained by the Quantitative Nuclear Magnetic Resonance technique, however the resonance technique can be used in the forensic area as a complement to mass spectrometry, in addition to demonstrating its viability in view of the results obtained.

Keywords: Rebites; Stimulants; Caffeine; qNMR

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LISTA DE SIGLAS E ABREVIATURAS

ANVISA	Agência Nacional de Vigilância Sanitária
CID	*Collision-induced Dissociation*
CNH	Carteira Nacional de Habilitação
CTB	Código Brasileiro de Trânsito
DAD	Arranjo de diodos
EDS	*Energy dispersive X-ray spectroscopy*
EMAR	Espectrometria de Massas de Alta Resolução
ESI	*Electrospray ionization*
FEG	*Field Emission Electron Guns*
HPLC	*High-performance liquid chromatography*
HRMS	*High Resolution Mass Spectrometry*
INCT	Instituto Nacional de Ciência e Tecnologia Forense
LSD	Dietilamida do Ácido Lisérgico
m/z	Massa/carga
MDA	3,4-metilenodioxianfetamina
MDEA	Metildietanolamina
MDMA	3,4-metilenodioximetanfetamina
MET	Microscopia Eletrônica de Transmissão
MEV	Microscopia Eletrônica de Varredura
qNMR	*Quantitative Nuclear Magnetic Ressonance*
Q-TOF	Quadrupolo-tempo de voo
REM	Radiação Eletromagnética
RMN	Ressonância Magnética Nuclear
SNC	Sistema Nervoso Central
SNP	Sistema Nervoso Periférico
THC	Tetrahidrocanabinol
TOF	*Time of flight*
W	Tungstênio

SUMÁRIO

INTRODUÇÃO

Droga é um termo genérico que define qualquer substância, seja natural, semissintética ou sintética, que pode causar dependência ao usuário. As drogas podem ser classificadas de diversas formas, seja por legalidade, mecanismo de ação ou produção. Assim, quanto a legalidade pode ser dividida em dois grupos, lícitas ou ilícitas. Drogas lícitas são aquelas legalizadas, ou seja, tem livre comércio, produção e consumo, como o cigarro, bebidas alcóolicas e estimulantes como a cafeína. Em contrapartida, as drogas ilícitas como maconha, ecstasy, cocaína, entre outros, tem seu comércio, produção e consumo vedados legalmente (GLIDIZ, 2016). No Brasil, essas substâncias são regulamentadas pela Agência Nacional de Vigilância Sanitária (ANVISA) através da Portaria nº 344 de 1998.

No Brasil, o transporte rodoviário é o mais utilizado levando a uma ampla malha viária e com isso, o aumento da probabilidade de acidentes de trânsito. Assim, segundo o Ministério da Infraestrutura, os caminhoneiros estão envolvidos em 4,41% dos acidentes de trânsito e aproximadamente 10,0% das mortes causadas por esses acidentes no Brasil (INFRAESTRUTURA, 2021).

O uso de substâncias psicoativas tem grande impacto nesses números, pois aumentam o risco de acidentes fatais cinco vezes em comparação com motoristas não usuários (OPAS, 2019a). A pressão econômica tem causado um aumento significativo no uso de estimulantes por motoristas profissionais, que geralmente trabalham por longas horas devido aos prazos extremamente curtos de entregas de mercadorias (TAKITANE, 2013). Entre estes compostos podemos destacar o uso recorrente de estimulantes como anfetamínicos, cocaína e seus derivados, como indicado por Bombana et al (2017), o estudo mostrou que de 764 caminhoneiros entrevistados, 40 usaram uma ou mais substâncias ilícitas. As substâncias identificadas foram: em 21 amostras foi detectado cocaína, enquanto 16 foi identificado anfetamina e em 8 foi identificado THC. Ainda, das 40 amostras avaliadas, 5 amostras apresentaram mais de uma substância, as quais foram: cocaína e THC, cocaína e anfetamina, anfetamina e THC.

O estudo foi realizado por 5 dias nas rodovias federais do estado de São Paulo entre 9h e 16h durante março de 2014 e março de 2015. Já os compostos lícitos, o estimulante mais utilizado é a cafeína, a qual provém de medicamentos comerciais e em drogas ilegais como alguns anfetamínicos e "rebites", este último pode ser encontrado na

maioria dos postos de gasolina ao longo das rodovias brasileiras (BRASIL, 2011; GJERDE, 2014).

Os rebites são compostos sem o controle de qualidade necessário, e podem conter as mais diversas substancias, um exemplo disso é o estudo realizado por Takitane (2013) onde 390 caminhoneiros em 3 rodovias de 3 cidades do estado de São Paulo aceitaram participar do estudo. As amostras deram positivo para cocaína, benzoilecgonina, THC-COOH, femproporex e anfetamina e, além disso, alguns motoristas alegaram fazer uso de um dos medicamentos analisados pelo nosso estudo, o Desobesi-M®.

Para qualificar e/ou quantificar essas substâncias, são desenvolvidos métodos analíticos com seletividade e sensibilidade adequados, que podem ser tanto presuntivos quanto confirmatórios. Neste sentido, os testes confirmatórios são testes mais complexos que envolvem técnicas como a cromatografia, a espectrometria de massas e a ressonância magnética nuclear (CARLIN; DEAN, 2013). Neste sentido, o estudo citado anteriormente, Takitane (2013) utilizou um cromatógrafo a líquido com detector de arranjo de diodos (DAD) para quantificar principalmente anfetamina em urina de caminhoneiros que faziam o uso de rebites.

Desta forma, este trabalho busca identificar compostos ativos não-alvo, não esperados, em amostras conhecidas como “Rebites” utilizadas por motoristas profissionais de cargas pesadas no Brasil. Dessa forma, utilizou-se a técnica de EMAR para uma visão geral do conteúdo dessas drogas, posteriormente foi utilizada a técnica de RMN para quantificar o principal ativo encontrado nos comprimidos, a cafeína, a qual foi confirmada por HPLC-UV-vis. Ainda, analisamos os fármacos por Microscopia Eletrônica de Varredura (MEV) com análise elementar por Espectroscopia de raios X por dispersão em energia. (EDS), para verificar a aplicação dessa análise direta na determinação desses fármacos em comprimidos.

OBJETIVOS

1.1 OBJETIVO GERAL

Realizar um *screening* geral por espectrometria de massas de alta resolução (EMAR) dos compostos ativos presentes em amostras de rebites coletados em diferentes pontos do país. Quantificar o principal ativo, cafeína, por Ressonância Magnética Nuclear

(RMN), realizando a quantificação também por Cromatografia a Líquido de Alta Eficiência (CLAE), a fim de verificar a veracidade do resultado obtido por RMN.

1.2 OBJETIVOS ESPECÍFICOS

- Identificar os compostos ativos não-alvo presentes em amostras de rebites coletados em diferentes regiões do país através da técnica de EMAR;
- Quantificar o composto majoritário não-alvo (cafeína) através da análise de RMN modo quantitativo (RMNq);
- Avaliar a quantidade deste mesmo ativo por Cromatografia a Líquido de Alta Eficiência com detector – UV-vis (CLAE-UV-vis);
- Avaliar a possibilidade de uso de microscopia com análise elementar FEG-EDS para qualificar os compostos presentes nos rebites avaliados;

REFERENCIAL TEÓRICO

1.3 TRANSPORTE RODOVIÁRIO NO BRASIL

As rodovias são fundamentais para a economia brasileira, pois é a principal via para o transporte de cargas, onde cerca de 75% de todas as mercadorias são transportadas, já que a malha rodoviária federal possui, atualmente, uma extensão total de 75,8 mil km (BELAN *et al.*, 2017; INFRAESTRUTURA, 2019a; INFRAESTRUTURA, 2021a). O relatório estatístico fornecido pelo Departamento Nacional de Trânsito do Brasil indicou que em 2021 a frota de veículos automotores atingiu 111 milhões, onde 192,4 mil correspondem a automóveis de transporte de carga (INFRAESTRUTURA, 2021b). Em consequência disso, há um aumento na probabilidade de acidentes de trânsito, que até setembro de 2021 atingiu um total de 3 milhões de acidentes (CNT, 2019; INFRAESTRUTURA, 2021b). Suas principais causas estão relacionadas à velocidade, ausência do uso de equipamentos de segurança e condução perigosa sob efeito de substâncias psicoativas (CYPRIANO, 2019). Além disso, muitas vias rodoviárias apresentam uma péssima condição, fator que contribui para o aumento de acidentes de

trânsito (BELAN *et al.*, 2017).

Segundo o Ministério da Infraestrutura, os caminhoneiros estão envolvidos em 4,41% dos acidentes de trânsito e aproximadamente 10,0% das mortes causadas por acidentes no Brasil. O uso de substâncias psicoativas tem grande impacto nesses números, pois aumentam cinco vezes o risco de acidentes fatais em relação aos motoristas não usuários (OPAS, 2019a). A lei 13.103 de 2015 do Código Brasileiro de Trânsito (CTB) exige que motoristas profissionais, referentes as categorias C, D e E da carteira nacional de habilitação (CNH) realizem exames toxicológicos para a renovação da mesma. Para esses exames são utilizadas amostras de cabelo, pelo ou unhas com análise retrospectiva mínima de noventa dias e visam identificar o uso ativo ou não de drogas psicoativas a fim de reduzir seu consumo por esses profissionais (INFRAESTRUTURA, 2019b).

A pressão econômica tem causado um aumento significativo no uso de estimulantes por motoristas profissionais, que costumam ter uma jornada de trabalho estendida (TAKITANE, 2013). Isso se deve aos prazos curtos de entrega de mercadorias levando a jornadas de trabalho exaustivas e a uma baixa remuneração. Em decorrência disso, o profissional acaba tendo uma baixa qualidade de vida e, principalmente, de saúde (SOUZA et al., 2016).

1.4 DROGAS DE ABUSO X TRANSPORTE RODOVIÁRIO

A portaria n° 344 de 1998 define “droga” como qualquer substância com finalidade medicamentosa ou sanitária, além disso define entorpecentes como substância que pode causar dependência psíquica ou física. Já a lei 11.343 de 2006 define “droga” como quaisquer substância ou produto que possa causar dependência. A partir disso, anfetaminas e seus derivados estão inseridos na lista A3 de substâncias psicotrópicas da portaria nº 344.

As drogas ilícitas podem ser classificadas conforme suas ações no sistema nervoso central (SNC), onde são divididas em depressora, estimulante e perturbadora. Drogas que diminuem tanto a atividade motora quanto a mental são denominadas de depressoras, como álcool, benzodiazepínicos e barbitúricos. Já aquelas que aumentam a atividade de certos sistemas neurais são denominadas de estimulantes, como anfetamínicos e cocaína. E, por fim, aquelas que prejudicam o funcionamento cerebral levando a alucinações e

delírios são denominadas de perturbadoras, como LSD e psilocibina (MAISTO; GALIZIO; CONNORS, 2017; SILVA et al., 2018).

Dessa forma, as drogas de abuso são substâncias que alteram tanto o humor quanto percepções sensoriais e afetam o encéfalo. Assim, a ingestão frequente causa dependência e aumenta a tolerância do organismo aos efeitos, levando a necessidade de doses mais altas de substância para produção do efeito desejado (RUSSELL; NEWMAN; BLAND, 1994; HALL; FARRELL, 2008; GOSSOP, 2011). Ainda, um dos fatores que geram abuso de drogas são os efeitos imediatos gerando gratificação instantânea. Além disso, há outros fatores como: uso recorrente da substância em situações de risco a segurança física, impossibilidade do usuário a cumprir suas obrigações e problemas legais recorrentes (HEAL *et al.*, 2013; RUSSELL; NEWMAN; BLAND, 1994; HALL; FARRELL, 2008; GOSSOP, 2011).

A partir disso, os estimulantes são uma classe de drogas que tem como característica diminuir a necessidade de dormir e o apetite, intensificar a capacidade de concentração, produzir sensações de energia e euforia, além de efeitos similares aos da adrenalina relacionados a pulsação, respiração e temperatura corporal. Estão inseridos nessa classe a cocaína e derivados, anfetamínicos, supressores de apetite e fármacos estimulantes prescritos para tratamento do transtorno de hiperatividade com déficit de atenção. Com isso, embora a nicotina e a cafeína tenham propriedades estimulantes, não são considerados drogas ilícitas por apresentarem diferentes mecanismos neuroquímicos. Além de seus usos não estarem associados ao padrão agudo de superdosagem e transtornos psiquiátricos temporários induzidos pelos outros estimulantes. Contudo o "vício" na nicotina foi comprovado quando a substância é usada de modo contínuo levando a sérias consequências médicas, porém não são consequências psiquiátricas (RUSSELL; NEWMAN; BLAND, 1994; HALL; FARRELL, 2008; GOSSOP, 2011).

Ao redor do mundo, drogas ilícitas como cocaína, anfetamina, tetrahidrocanabinol (THC), opiáceos e metadona vem sendo relatados em análises de fluído oral. Em países como EUA, Brasil, Noruega, Itália, Espanha e Hungria relatam o uso majoritário de anfetamina, canabinoides, cocaína e opiáceos (KNOW, 2019). Um estudo realizado por Know (2019) em motoristas sob influência de drogas na Ásia, Europa e EUA em matrizes biológicas comuns e alternativas nos anos de 2013 a 2018, relata uso comum de oito drogas de abuso. Dentre as drogas de abuso relatadas, a anfetamina é mais difundida nos EUA, Turquia e alguns países da Europa, como Bélgica, Itália, Finlândia, Noruega, Dinamarca, Alemanha, Suíça e Reino Unido. Enquanto a metanfetamina é mais difundida

na Ásia, sendo relatada em países como China, Irã e Coréia do Sul, além disso os países da Europa que diferem do uso de anfetamina são Reino Unido e Dinamarca, pois não apresentam a metanfetamina como droga de abuso comumente utilizada (KNOW, 2019). Já no Brasil, as drogas de abuso mais comuns utilizadas por motoristas profissionais são anfetamínicos e cocaína, além de outros estimulantes lícitos como a cafeína. Estes são oriundos de medicamentos comerciais, tráfico de drogas e drogas ilícitas como “rebites”, que podem ser encontradas na maioria dos postos de gasolina ao longo das rodovias brasileiras (BRASIL, 2011; GJERDE, 2014).

1.4.1 Anfetamínicos

Os estimulantes do tipo metilfenetilamina são divididas em drogas como anfetamina, metanfetamina e seus análogos e drogas do tipo "ecstasy" como MDMA, MDA e MDEA (DE-CAROLIS *et al.*, 2015). Anfetamínicos é a terceira classe de drogas mais utilizada no mundo, com 29 milhões de usuários de alguma substância registrados no ano de 2017 (CYPRIANO, 2019).

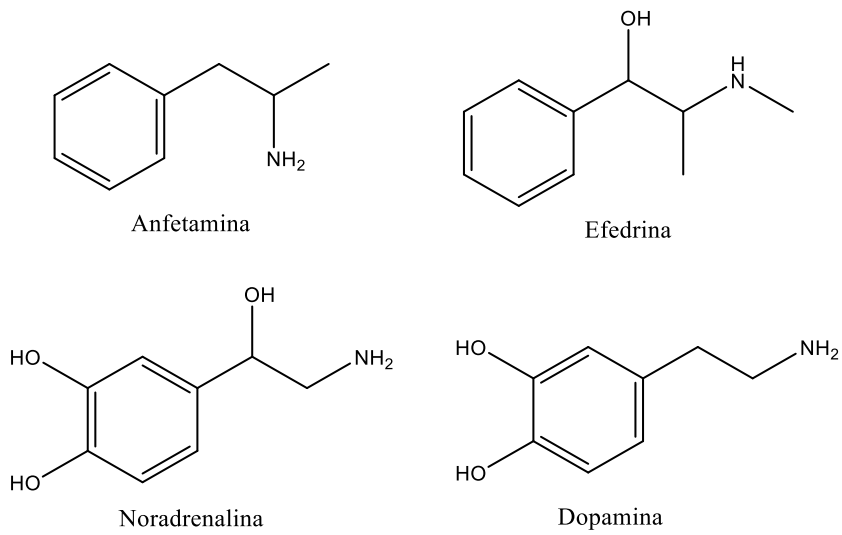
A anfetamina, forma racêmica alfa-metilfenetilamina, foi descoberta em 1910 por Barger e Dale, porém só foi sintetizada em 1927 pelo químico G. A. Alles, o qual buscava um substituto para a efedrina onde sua sintetização fosse mais simples e barata. A empresa Smith, Kline and French registrou com o nome comercial de Benzedrine®, a qual foi colocada no mercado em 1935 para tratamento de narcolepsia, depressão, Parkinson e outros distúrbios (HEAL *et al.*, 2013).

Em 1946 na Segunda Guerra Mundial, a anfetamina foi utilizada tanto pelos aliados como pelo Eixo, com o intuito de manter as tropas em um estado de alerta constante. Ainda, estima-se que 150 milhões de comprimidos foram fornecidos aos militares britânicos e americanos durante o conflito (HEAL, 2013). Atualmente as anfetaminas é a segunda classe de drogas mais abusadas na Europa, depois da cannabis (STEINKELLNER *et al.*, 2011).

Os anfetamínicos apresentam pré-requisitos físico-químicos que são característicos para os transportadores de recaptação de monoamina, os quais são: a sua conformação planar, tamanho molecular semelhante às monoaminas, a presença de um anel aromático e um nitrogênio na cadeia lateral arila. Dessa forma, a anfetamina é agonista do SNC e sua principal ação é aumentar as concentrações sinápticas de neurotransmissores no SNC, principalmente da noradrenalina e da dopamina de forma

indireta. A Figura 1, demonstra a clara semelhança entre as estruturas químicas da anfetamina, efedrina e dos neurotransmissores, noradrenalina e dopamina (HEAL *et al.*, 2013; STEINKELLNER *et al.*, 2011).

Figura 1 - Estruturas químicas da anfetamina, efedrina, noradrenalina e dopamina



As anfetaminas exercem seus efeitos agudos tanto no SNC quanto em tecidos periféricos, onde o desfecho clínico depende da dose administrada. Assim, o usuário experimenta alguns efeitos positivos como um aumento do estado de excitação, euforia, aumento da energia e falação, mas também efeitos negativos como ansiedade, paranoia ou alucinações auditivas e visuais. Já os efeitos periféricos das anfetaminas incluem aumentos na frequência cardíaca, pressão arterial, taxa de respiração, temperatura corporal, ativação psicomotora e redução do apetite (STEINKELLNER *et al.*, 2011).

Em contrapartida da anfetamina, a metanfetamina foi sintetizada pela primeira vez em 1919 no Japão. E sabe-se que, na Segunda Guerra Mundial, os pilotos kamikazes japoneses receberam altas doses de metanfetamina antes de suas missões suicidas (DE-CAROLIS *et al.*, 2015). Além disso, há indícios de que Hitler fazia uso da droga 8 vezes ao dia (MEHLING, 2008).

A metanfetamina é um óleo volátil insolúvel e incolor, já como sal de cloridrato é um pó branco cristalino amargo que pode ser facilmente dissolvido em água ou álcool. Seu uso mais comum é a ingestão de comprimidos, embora possa ser inalada ou injetada.

Ainda, a metanfetamina em pó é geralmente dissolvida em bebida e é conhecida por: *Crank, Crypto, Fire, Meth, Speed*. Já para ser fumada, é necessário ser purificado em um grande cristal e é conhecida por *Crystal Meth, Crystal Glass, Ice* (DE-CAROLIS *et al.*, 2015).

Alguns efeitos da metanfetamina quando fumado ou injetado levam ao "*rush*", semelhante ao da cocaína, aonde essa fase está associada à taquicardia, sudorese e aumento da pressão arterial por cerca de 30 min, diferente da fase do "*high*" que pode durar várias horas levando a efeitos como arrogância e hiperatividade. Ainda, após essas duas fases, há a fase do "*binge*", a qual é um longo período de 3 a 15 dias durante o qual o usuário tenta manter os efeitos da fase "*high*" através da ingestão de doses cada vez maiores. Quando essas doses já não causam os efeitos desejados, o usuário entra na fase do "*tweaking*" ou "*itch*", levando a efeitos como hiperestesia da pele e alucinações táteis. O usuário sofre de psicose, insônia, mania de perseguição e negligencia a higiene pessoal. Por fim, após um ou dois meses, o usuário chega a fase da abstinência, levando a efeitos de depressão, disforia, fadiga e ideação suicida (DE-CAROLIS *et al.*, 2015).

Como a metanfetamina tem meia-vida de 12 h, seus efeitos duram mais do que os da cocaína (WINSLOW; VOORHEES; PEHL, 2007). Outros efeitos causados pela metanfetamina estão descritos na Quadro 1.

Quadro 1 - Sintomas e sinais clínicos da metanfetamina

Toxicidade	**Possíveis sintomas e sinais clínicos**
Suave	Bruxismo, diarreia, dor abdominal, dores de cabeça, hiperreflexia, inquietação, insônia, irritabilidade, midríase, náusea, palpitação, rubor ou palidez, sudorese, tremores, trismo, vomito, xerostomia.
Moderado	agressividade, alucinações, ansiedade, confusão, desidratação, dispneia, dores no peito, hiperatividade, hipertensão, pirexia leve, rigidez muscular, taquicardia, taquipneia.
Severo	Alucinações, coma, convulsões, delírio, disritmia cardíaca, falência renal associada a rabdomiólise, hiperpirexia, hipertensão ou hipotensão, paranoia.
Potencialmente fatal	Acidente vascular cerebral, podendo ocorrer hemorragia cerebral, convulsões repetidas, edema cerebral com compressão do tronco cerebral secundária a hipóxia ou hiponatremia, fibrilação ventricular, hipertermia podendo ocorrer coagulação intravascular disseminada, infarto do miocárdio, insuficiência cardíaca aguda.

Fonte: De-carolis *et al.* (2015)

Como a metanfetamina é absorvida rapidamente pelo trato gastrointestinal e eliminada principalmente pela urina, para casos de overdose é necessário a acidificação da urina do usuário para evitar que o mesmo venha a óbito (METAMFETAMINE, 2020; DE-CAROLIS *et al.*, 2015).

Diferente da anfetamina e da metanfetamina, o ecstasy, também chamado de MDMA, foi inicialmente produzido como inibidor de apetite pela indústria farmacêutica Merck no ano de 1914. (ROMÃO, 2010; OLIVE, 2004). Já nos anos 60, o ecstasy era utilizado para elevar o ânimo de pacientes psicoterápicos (OLIVE, 2004).

Pode causar efeitos como estado de alerta, euforia e aumento da sociabilização. Além disso, há os efeitos negativos, tais como a disfunção do sistema imunológico, a diminuição da capacidade de funcionamento do fígado e o aumento da temperatura corporal. Ainda, seus usuários podem permanecer sob efeito durante, em média, 8 h. (GIL, GIMENEZ E SAUAEZ, 2014; OLIVE, 2004). O abuso de MDMA pode levar à morte, porém intoxicações fatais são eventos raros que, na maioria dos casos, está associada a utilização de mais de uma classe de drogas ilícitas. O abuso do ecstasy leva a efeitos como hipertermia aguda, hiponatremia, colapso cardiovascular e rabdomiólise (STEINKELLNER *et al.*, 2011).

Segundo o III Levantamento Nacional sobre o Uso de Drogas pela População Brasileira realizado pela Fundação Oswaldo Cruz no ano de 2015, apenas 235 entrevistados utilizaram MDMA/ecstasy nos últimos 12 meses entre pessoas de 12 a 65 anos. Já a prevalência desse uso é de 0,2%. Ainda, segundo o Levantamento o número de consumidores de MDMA/ecstasy homens é maior que mulheres, sendo 150 homens entrevistados e 85 mulheres (FUNDAÇÃO OSWALDO CRUZ, 2017).

A anfetamina quando ingerida via oral é menos prazerosa, ou seja, os efeitos positivos são inferiores quando comparados com a metanfetamina e cocaína, isso se deve porque a cocaína quando cheirada ou fumada tem seu "*high*" potencializado por entrar mais rapidamente no cérebro. Diferente da metanfetamina que entra mais lentamente no cérebro, adiando seus efeitos em 10 a 15 min quando comparados a cocaína. E apesar de não ter muito estudo sobre o sulfato de d-anfetamina pode-se prever através de suas propriedades físico-químicas que sua absorção seria ainda mais lenta que a metanfetamina (HEAL *et al.*, 2013).

1.4.2 Cafeína

A cafeína é um estimulante da classe das metilxantinas que está presente em analgésicos, chás, cafés e refrigerantes a base de cola (CAFFEINE, 2022). Acredita-se que a cafeína seja o psicoestimulante mais consumido em todo o mundo sendo ingerido majoritariamente como café, o qual é a bebida mais popular depois da água sendo consumindo diariamente 1,6 bilhões de xícaras, aproximadamente. O café provém da planta *Coffea arabica*, originária da Etiópia, apesar disso foi o Iêmen o primeiro país a cultivar a planta. Sabe-se que a cafeína não é essencial para a planta, servindo como um mecanismo de defesa se assemelhando a um pesticida já que é tóxica para vários insetos e animais, principalmente herbívoros (CAPPELLETTI *et al.*, 2015).

Apesar da cafeína ser legalmente comercializada como o álcool e o tabaco, sua venda em bebidas ou comprimidos não é controlada ou restrita. Contudo, em 2004 o Comitê Olímpico Internacional colocou a cafeína em sua lista de substâncias proibidas. E, assim como anfetamínicos, a cafeína tem se mostrado eficaz em tratamentos experimentais em doenças como Alzheimer e Parkinson (CAPPELLETTI *et al.*, 2015).

A cafeína, assim como anfetamínicos é agonista do SNC, estimula o SNC, aumentando o estado de alerta e podendo causar inquietação e agitação. Além disso, é

agonista do SNC através do receptor adenosina que, por sua vez, afeta a liberação de neurotransmissores como a dopamina, assim como anfetamínicos. Além desse, há outros neurotransmissores envolvidos, como norepinefrina, serotonina, acetilcolina, entre outros. Contudo, diferente dos anfetamínicos a cafeína apresenta ainda dois mecanismos possíveis de ação no SNC, sendo eles: o efeito da metilxantina na mobilização do cálcio intercelular e a capacidade da metilxantina de inibir as fosfodiesterases. Ambos mecanismos necessitam de uma concentração alta de cafeína no organismo, considerada tóxica para seres humanos (FIANI *et al.*, 2021).

O consumo de cafeína foi classificado em 3 categorias, onde: usuários de cafeína baixa consomem menos que 200 mg/dia, já usuários moderados de cafeína consomem de 200 a 400 mg/dia e, por fim, usuários de cafeína alta consomem mais de 400 mg/dia. Assim, a overdose fatal aguda em humanos é equivalente a 150 a 200 mg/kg (CAPPELLETTI *et al.*, 2015; CAFFEINE, 2022).

Apesar de os efeitos da dependência da cafeína ainda estarem sob investigação indivíduos que consomem altas doses de cafeína frequentemente relatam sintomas de transtornos psiquiátricos, principalmente de ansiedade e humor, mas também alterações comportamentais. Os sintomas comuns da intoxicação por cafeína incluem ansiedade, agitação, inquietação, insônia, distúrbios gastrointestinais, tremores, taquicardia, agitação psicomotora e, em alguns casos, morte (CAPPELLETTI *et al.*, 2015).

A meia-vida da cafeína em um adulto é de aproximadamente 5 h, contudo várias características e condições podem acabar alterando essa estimativa. Um exemplo disso é que em fumantes a meia-vida pode ser reduzida em até 50%, enquanto em mulheres grávidas há um aumento para 15 h ou mais, principalmente no terceiro trimestre (DRUGBANK, 2022).

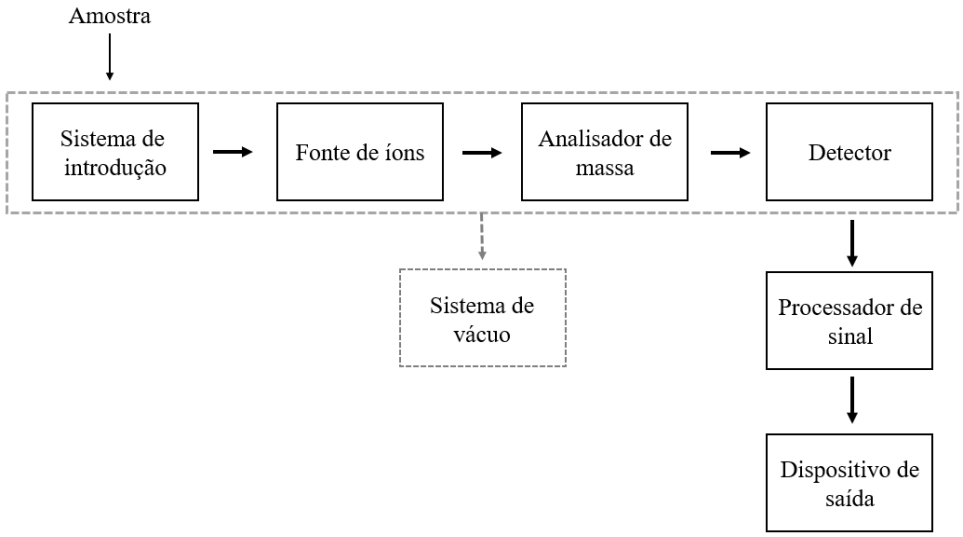
1.5 MÉTODOS ANALÍTICOS NA IDENTIFICAÇÃO DE DROGAS DE ABUSO

1.5.1 Espectrometria de massas

A espectrometria de massas é uma das técnicas mais utilizadas no âmbito forense, isso se deve ao breve tempo de análise, um preparo de amostra relativamente simples e, por fim, a quantidade de amostra utilizada para a realização da análise (ROMÃO, 2010).

Nessa técnica as colisões entre elétrons energéticos e as moléculas do analito fornecem a energia suficiente para as moléculas ficarem em seu estado excitado, permitindo que cada íon produzido seja ordenado com base na sua razão massa/carga (*m/z*) gerando um espectro de massa. (SKOOG; HOLLER; CROUCH, 2016; GIL, GIMENEZ E SAUAEZ, 2014). A Figura 2 representa um fluxograma de blocos que ilustra um espectrômetro de massas.

Figura 2 - Fluxograma de um espectrômetro de massas



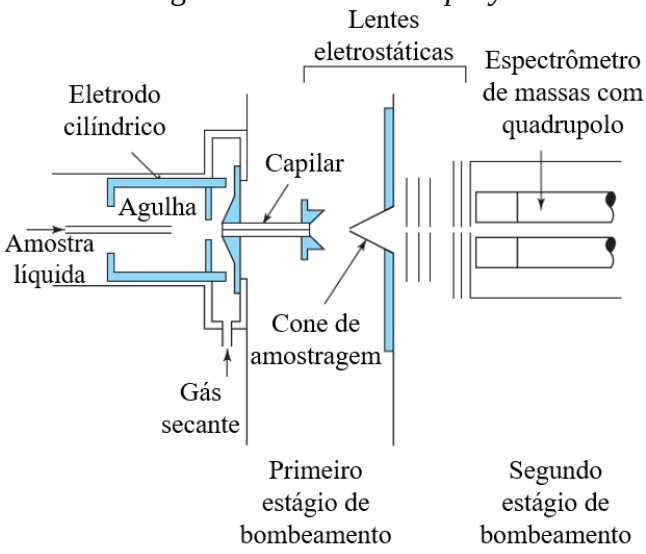
Fonte: Adaptado de Skoog, Holler e Crouch (2016).

As razões *m/z* podem servir para qualificar um composto específico, seja ele previsto ou não para estar presente naquela amostra. Para isso, uma quantidade de 10 a 1 ppm é inserida no equipamento através de métodos de separação como, por exemplo, cromatografia ou infusão direta, este último se dá através do auxílio de uma bomba de seringa. Em seguida, as amostras passam através de uma fonte de ionização, responsável em converter a amostra em um feixe de íon positivos, assimilando carga via adição de um hidrogênio, de *addutos*, entre outros. Ainda, é possível que a amostra seja "carregada" negativamente através da perda de um hidrogênio, ou de outras formas (SKOOG; HOLLER; CROUCH, 2016).

Como se faz necessário que os íons do analito estejam no estado gasoso para a realização da análise, o processo de ionização é fundamental, já que irá influenciar no método utilizado para o ensaio. Dessa forma, a ionização por eletronebulização, do inglês *Electrospray ionization (ESI)*, é uma técnica que utiliza uma fonte de dessorção, onde a

amostra líquida ou sólida em solução será convertida diretamente para seu estado gasoso. Com isso, essa técnica pode ser aplicada tanto para amostras não-voláteis, quanto para amostras termicamente instáveis (SKOOG; HOLLER; CROUCH, 2016). No sistema *eletrospray*, a amostra é bombeada por uma agulha capilar para dentro de uma câmara aquecida em pressão próxima a atmosférica em forma de gotículas. Estas são carregadas devido a presença do eletrodo cilíndrico que envolve a agulha, após passam por um tubo capilar. Assim entram em contato com o gás secante, geralmente nitrogênio, o qual tem a função de evaporar o solvente ainda presente nas gotículas. Ao passo que a densidade dessas gotículas cresce, ocorre a dessorção dos íons no gás ambiente (SKOOG; HOLLER; CROUCH, 2016). A fonte *eletrospray* está representada na Figura 3.

Figura 3 - Fonte *eletrospray*



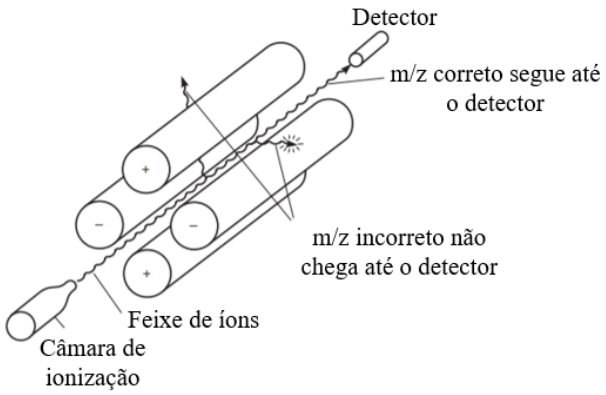
Fonte: Adaptado de Skoog, Holler e Crouch (2016).

Em seguida, esses íons são acelerados para dentro do analisador de massa, o qual separa os íons conforme sua razão *m/z*. O equipamento utilizado apresenta dois separadores de massas sequenciais do tipo quadrupolo e tempo de voo, respectivamente.

O analisador de massa do tipo quadrupolo é o mais comumente utilizado em espectrometria de massas para análise de compostos alvo, os quais espera-se encontrar na amostra. Isso se deve por ser mais compacto que outros analisadores e seu tempo de varredura curto, sendo geralmente menor que 100 ms (SKOOG; HOLLER; CROUCH, 2016). Esse analisador contém quatro barras sólidas, conforme apresentado na Figura 4, nas quais são aplicadas uma corrente contínua e uma radiofrequência gerando um campo

eletrostático oscilante entre as barras. Os íons com oscilações instáveis são aqueles que apresentam uma razão *m/z* muito pequena ou muito grande, logo a trajetória deles os levará até uma das quatro barras. Já os íons com oscilações estáveis são aqueles que apresentam a faixa correta de razão *m/z*, a qual é imposta no software pelo operador, logo sua trajetória os levará até o detector (PAVIA et al., 2015).

Figura 4 - Analisador de massa com quadrupolo



Fonte: Adaptado de Pavia et al. (2015).

O analisador por tempo de voo, do inglês *time of flight* (TOF), traz uma robustez e simplicidade ao espectrômetro de massas. Esse analisador baseia-se na velocidade de dois íons, com massas diferentes, que chegarão ao detector com tempos diferentes apesar de apresentarem a mesma energia cinética e terem sido criados no mesmo instante. Esses íons são acelerados dentro um tubo de trajetória livre através de um pulso de campo elétrico com variação entre 10^3 a 10^4 V. Além disso, os tempos de voo comuns variam de 1 a 30 μs (SKOOG; HOLLER; CROUCH, 2016).

Tendo isso em vista, estes dois separadores de massas em sequência geram resultados de alta resolução, a qual apresenta uma faixa entre 10.000 a 100.000. Dessa forma, analisadores EMAR podem melhorar o processo de identificação e confirmação de moléculas a partir de diversos parâmetros como massa exata, razão isotópica e perfil de fragmentação. Com isso, é possível obter uma confirmação do composto desejado sem interferência (KELLMANN *et al.*, 2009).

Assim, esta análise necessita de um sistema de vácuo para criar baixas pressões, geralmente de 10^{-4} a 10^{-8} torr, pois dessa forma as partículas carregadas serão aniquiladas ao interagirem com componentes da atmosfera. Após têm-se o processador de sinal e o

dispositivo de saída, os quais separam os íons gerados em um padrão de espectro de massa (SKOOG; HOLLER; CROUCH, 2016).

Contudo há ainda dois fatores que devem ser avaliados, o primeiro são os padrões de fragmentações. Como a molécula entra em um estado excitado, muitas vezes esse estado não é estável e acaba quebrando-se em fragmentos menores, o qual geralmente é alcançado através do aumento da energia de ionização. Em um espectro, geralmente não é possível analisar todos os picos presentes, pois normalmente há a presença de ruídos. Dessa forma, busca-se padrões de fragmentação principalmente do pico majoritário, também denominado de íon molecular, onde é possível a identificação de grupos funcionais presentes (SKOOG; HOLLER; CROUCH, 2016).

E por fim, outro fator a ser avaliado é o erro de exatidão de massa. Geralmente, para EMAR com ionização por ESI e separação por Q-TOF, um valor de erro deve ser inferior a 10 ppm para a confirmação da fórmula elementar do composto (BRISTOW; WEBB, 2003). Para minimizar erros nessa medida se faz necessário garantir que o íon alvo esteja completamente livre de íons interferentes para que não ocorra alterações na massa do pico (ROMERO-GONZÁLEZ; FRENICH, 2017). Essa verificação pode ser determinada a partir da Equação 1 (KELLMANN *et al.*, 2009).

$$\text{Erro (ppm)} = \frac{m_l - m_e}{m_e} \text{ x}10^6 \qquad (1)$$

Onde m_l é a massa obtida experimentalmente, enquanto m_e é a massa exata.

1.5.2 Ressonância Magnética Nuclear

A ressonância magnética nuclear (RMN) é uma técnica espectroscópica analítica que se baseia em absorção e reemissão de radiação eletromagnética (SILVA, 2018). Essa teoria teve seu início no ano de 1930 pelo alemão Gorter, pois o mesmo obteve um resultado negativo em um experimento de detecção do núcleo magnético (CROCOLI, 2019). Em seguida em 1945 dois grupos de pesquisas distintos, Edward Purcell e Felix Bloch, avaliaram o fenômeno de ressonância em líquidos e sólidos (SERRANO, 2019). Contudo, foi somente em 1963 que se utilizou a técnica de RMN quantitativo através da

preparação de três analitos diferentes por parte de Hollis e vinte e seis moléculas orgânicas por parte de Jungnickel e Forbes (LEITE, 2013; CROCOLI, 2019).

Além disso, é uma técnica relativamente nova, porém bem estabelecida nas mais diferentes áreas de aplicações, levando com que ela seja um dos procedimentos mais amplamente utilizados. Recentemente, a técnica quantitativa por RMN, também conhecida por q-NMR, tem sido bastante difundida na área farmacêutica e de controle de qualidade dos fármacos (NASCIMENTO, 2018). A RMNq possui vantagens muito importantes frente às outras técnicas, sendo elas:

I. curto tempo de análise;
II. fácil preparo de amostra;
III. a amostra não entra em contato com o equipamento, facilitando a manutenção do mesmo;
IV. técnica não destrutiva;
V. como o equipamento se mantém estável por mais tempo, não se faz necessário a calibração tão frequente quanto outras técnicas,
VI. a possibilidade de determinar mais de um analito em uma mistura, além de possibilitar a quantificação desses analitos uma vez que a magnitude do sinal gerado é diretamente proporcional ao número de hidrogênios do analito.

A espectroscopia de RMN se baseia nas propriedades magnéticas do núcleo atômico, baseadas, por sua vez, nos conceitos de spin nuclear e momento magnético do núcleo. Assim, para que um determinado isótopo possa ser analisado por RMN é necessário que este tenha: massa ímpar ou número atômico ímpar ou ambos, isso é denominado como propriedade de spin. Contudo, as aplicações de RMNq a núcleos distintos do 1H são limitadas devido à baixa sensibilidade ou baixa abundância natural dos mesmos (LEITE, 2013; CROCOLI, 2019).

Ainda, sabe-se que aproximadamente dois terços de todos os isótopos possuem momento magnético nuclear. Os núcleos dos isótopos com número quântico de spin I = ½ possuem apenas dois estados de energia, chamados de estado α ou “alinhado com o campo magnético principal” onde I = ½, estes estão no seu estado de menor energia quando exposto a um campo magnético estacionário externo (B0). Já o estado β ou “no sentido oposto ao estado α” onde I = -½, estes estão no seu estado de maior energia quando exposto a B0. Contudo, na ausência de um campo magnético externo esses dois estados

possuem o mesmo nível de energia e são ditos degenerados (SILVA, 2018; CROCOLI, 2019).

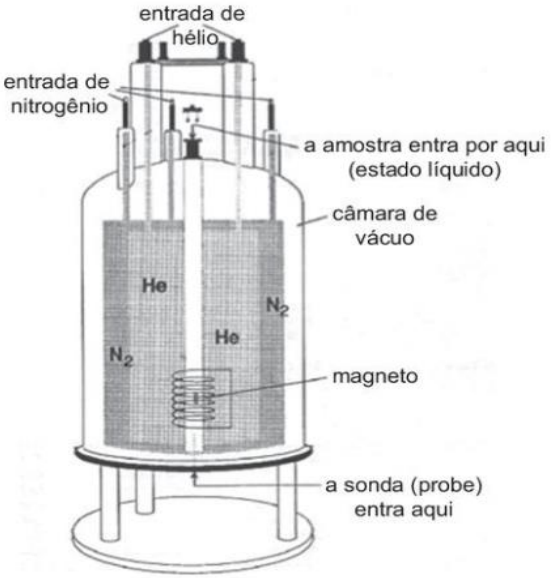
Além disso, haverá um pequeno excesso de núcleos no estado α, já que este estado é energeticamente mais favorável e é este excesso que é medido em RMN. Após, os spins excitados passam para a fase de relaxação, a qual pode ser definida como o tempo necessário para os spins retornem completamente ao seu estado fundamental de equilíbrio térmico (SERRANO, 2019; CROCOLI, 2019). Essa fase ocorre por dois mecanismos distintos e simultâneos: a longitudinal (T1) e a transversal (T2), é durante esse tempo que se observa o sinal induzido pela magnetização dos spins, o FID (*Free Induction Decay*), que contém todas as frequências espectrais sobrepostas. Nas análises de RMN de alta resolução se faz necessário aplicar a transformada de Fourier para que o sinal medido em tempo seja convertido em frequências (CROCOLI, 2019).

Assim, um espectro de RMN mostra aumento do deslocamento químico em ppm da direita para esquerda, indicando que os prótons menos blindados aparecerão mais à esquerda e os mais blindados à direita do espectro (LEITE, 2013).

A partir disso, o equipamento é composto por três grandes partes: o magneto, responsável por gerar o campo magnético B onde a amostra será inserida. Esse campo é gerado a partir de um solenoide supercondutor, o qual é necessário ser resfriado com hélio líquido para manter as condições de supercondutividade. Com isso, é necessário minimizar sua evaporação, uma vez que hélio liquido tem alto custo. Isso se dá através de um sistema de vácuo, seguido de outro reservatório com nitrogênio líquido e mais um sistema de vácuo. (NASCIMENTO, 2018).

Já o console, é o componente que controla o sistema do espectrômetro, onde se encontram alguns dispositivos como as fontes de radiofrequência que geram os pulsos, os amplificadores de sinal, os conversores analógico-digital, entre outros (NASCIMENTO, 2018). Por fim, temos o computador, de onde o operador realiza todas as tarefas para aquisição e processamento dos espectros para análises (NASCIMENTO, 2018). A Figura 5 representa um ressonância magnética nuclear.

Figura 5 – Ressonância Magnética Nuclear



Fonte: Nascimento (2018).

Ainda, o tubo onde a amostra desce é mantido à temperatura ambiente, porém é possível realizar experimentos em diferentes temperaturas. Não obstante, o RMN permite análises de substâncias nos estados sólido e líquido, onde as amostras devem ser dissolvidas em algum solvente deuterado, evitando assim que apareça um sinal de hidrogênio do solvente no espectro. Além disso, o deutério é importante para realizar o ajuste do campo magnético (*lock*) do equipamento. Ainda, como a técnica de RMN não é destrutiva e permite a recuperação da amostra, é recomendável solubilizar a amostra em solventes mais voláteis, para uma recuperação mais fácil da amostra (NASCIMENTO, 2018).

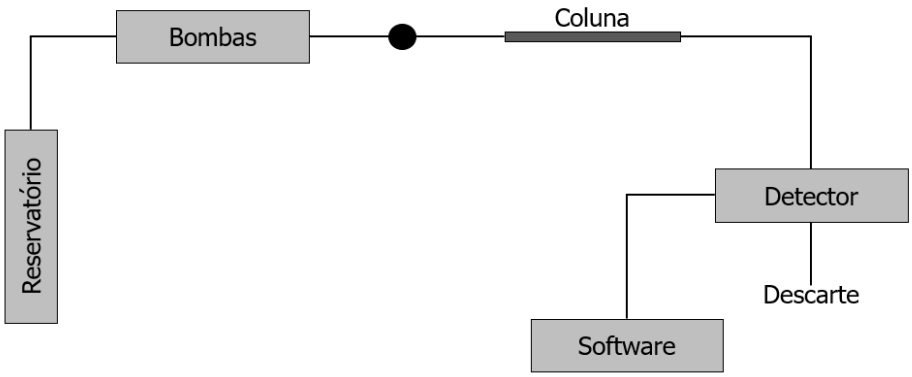
1.5.3 Cromatografia Líquida de Alta Eficiência

Cromatografia líquida de ala eficiência, do inglês *High-performance liquid chromatography* (HPLC) é a cromatografia mais versátil e amplamente utilizada, já que é usada para qualificar e quantificar compostos em uma variedade de materiais orgânicos, inorgânicos e biológicos. A popularidade da técnica se deve a sua sensibilidade, facilidade de automação, adequação à separação de compostos não voláteis ou termicamente

instáveis. Na cromatografia líquida, a fase móvel é um solvente líquido que contém a amostra como uma mistura de solutos (SKOOG; HOLLER; CROUCH, 2016).

Antigamente a cromatografia líquida (LC) era realizada em colunas de vidro com diâmetros de 10 a 50 mm, a fase estacionária era composta por partículas sólidas de comprimentos de 50 a 500 cm revestidas com um líquido adsorte. E para ter taxas de fluxo razoáveis, o tamanho da partícula do sólido era maior que 150-200 µm, dessa forma os tempos de separação eram longos, geralmente várias horas (SKOOG; HOLLER; CROUCH, 2016). A Figura 6 apresenta um fluxograma de blocos de um HPLC.

Figura 6 - Fluxograma de blocos de um HPLC



Fonte: Adaptado de Bayne, Carlin (2010).

No reservatório de solventes fica a fase móvel, podendo ser uma ou mais, a qual auxilia no transporte de compostos através da fase estacionária, efetuando a separação em componentes individuais. Além disso, é composta de soluções aquosas, de acetonitrila, de metanol e/ou tampões (BAYNE; CARLIN, 2010). Aqui é necessário ter cuidado com interferentes como bolhas e poeira, além de gases dissolvidos, pois podem levar a taxas de fluxo irreprodutíveis e propagação de banda, além de interferirem no desempenho dos detectores (SKOOG; HOLLER; CROUCH, 2016).

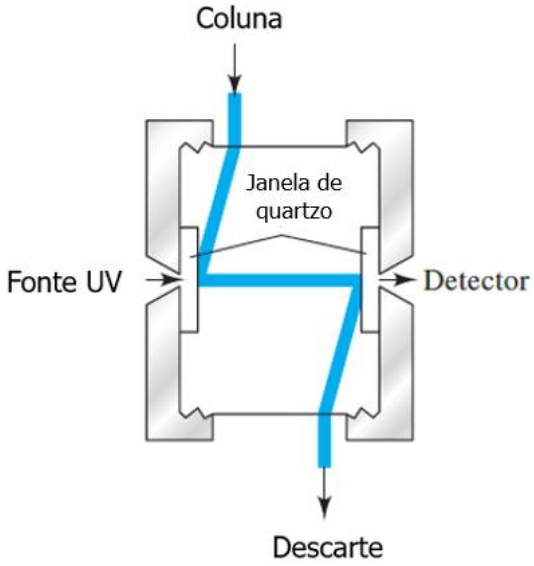
A bomba faz com que a fase móvel passe pela coluna em alta pressão e é monitorada e controlada pelo regulador de pressão. Há diferentes tipos de bombas, sendo as mais comuns as binárias e quaternárias, onde as binárias podem conduzir até duas soluções ao mesmo tempo e as quaternárias até quatro (BAYNE; CARLIN, 2010).

A coluna contém o material estacionário embalado e é onde será efetuada a separação da amostra em compostos individuais com o auxílio da fase móvel.

Normalmente a coluna opera em temperatura ambiente, mas pode ser aquecida ou resfriada, levando à estabilidade da coluna e separações de alta eficiência (BAYNE; CARLIN, 2010; SKOOG; HOLLER; CROUCH, 2016). Ainda, as colunas variam seu comprimento, tamanho de partícula de embalagem e diâmetro interno (BAYNE; CARLIN, 2010).

O tipo de detector vai variar dependendo da aplicação; no entanto, os sistemas mais utilizados são o conjunto diodo UV/Vis. Nesse detector, os analitos eluidos entram em uma célula de fluxo, essa é mantida no caminho de um feixe de radiação UV/Vis e o detector detecta analitos que absorvem nessa região do espectro eletromagnético. A fonte de radiação é uma lâmpada de filamento de deutério, mercúrio e/ou tungstênio. Um monocromador é usado para produzir uma única faixa estreita de UV ou radiação visível, a qual é passada através da amostra e a radiação transmitida é detectada por um fotomultiplicador. Ainda, os detectores UV/Vis podem apresentar comprimentos de onda fixos, onde os mais comuns são 220, 254, 436 e 546 nm, e comprimentos de onda variáveis (BAYNE; CARLIN, 2010). A Figura 7 mostra um detector UV/Vis.

Figura 7 – Detector UV/Vis



Fonte: Adaptado de Skoog, Holler e Crouch (2016).

O sistema de processamento de dados ou software permite a manipulação da resposta do sinal do detector em um formato legível na forma de um cromatógrafo, onde o eixo x representa o tempo e o eixo y representa a resposta do detector (BAYNE; CARLIN, 2010).

1.5.4 Microscopia Eletrônica de Varredura

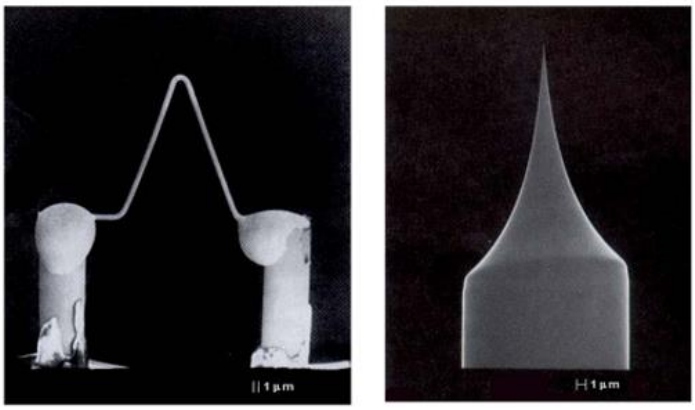
Existem dois tipos básicos de microscópios eletrônicos, porém com finalidades diferentes, sendo eles: o de transmissão (MET) e o de varredura (MEV). Enquanto que nos de transmissão os elétrons atravessam cortes ultrafinos da amostra e geram uma imagem bidimensional de seu interior sobre uma tela fluorescente. Nos de varredura os elétrons varrem apenas a superfície externa da amostra produzindo uma imagem tridimensional que é visualizada em um monitor acoplado ao microscópio. A partir dessa imagem tridimensional da amostra, é possível realizar pesquisas e avaliações dessas superfícies (PIRES; ALMEIDA; COELHO, 2014; MELO, 2018).

O MEV consiste em uma coluna ótico-eletrônica, uma unidade de varredura, uma câmara de amostra, um sistema de detectores e um sistema de visualização da imagem.

Na coluna ótico-eletrônica se encontram o canhão de elétrons, responsável por gerar os elétrons primários, as lentes condensadoras, responsável por reduzir o feixe de elétrons primários que são grosseiramente emitidos pelo canhão de elétrons. Há também as bobinas, que promovem a deflexão do feixe de elétrons primários no sentido horizontal e vertical sobre uma dada região da amostra, e ainda as bobinas que fazem as correções de astigmatismo. Toda a coluna deve estar sob vácuo durante a emissão do feixe de elétrons primários (MALISKA, 2022).

Há diversos tipos de canhão de elétrons, o que leva a variação da quantidade de corrente que as mesmas podem produzir, o tamanho da fonte, a estabilidade do feixe produzido e o tempo de vida da fonte. O modelo mais usado é formado por três componentes, chamado de tríodo, o qual é composto por um filamento de tungstênio (W), que serve como cátodo, um cilindro de Wehnelt e um ânodo. Contudo, há fontes melhores que a de tungstênio como a de emissão por efeito do campo (*Field Emission Electron Guns* - FEG), essa fonte é composta por um monocristal de tungstênio na forma de um fio com uma ponta extremamente fina, como apresentado na Figura 8 (MALISKA, 2022).

Figura 8 - Micrografia eletrônica de um filamento de W (a) e FEG (b)



(a) (b)

Fonte: Adaptado de Maliska (2022).

Como a ponta do filamento é muito fina, cerca de 100 nm ou menos, o campo elétrico fica extremamente alto. E enquanto a emissão realizada pelo filamento de tungstênio é de cerca de 3 A/cm2, a do FEG é de cerca de 105 A/cm2. Ainda, como a área do primeiro foco oriundo da fonte virtual é de aproximadamente 10 nm, a redução, conhecida também por demagnificação, do feixe de elétrons não precisa ser tão intensa resultando em uma resolução de 1 a 2 nm. Também, essas fontes podem ser aquecidas ou não, porém quando frias necessitam de um vácuo mais elevado. Em consequência da maior corrente e do menor feixe eletrônico, as fontes de emissão eletrostáticas produzem excelentes imagens. Contudo apresentam uma menor estabilidade do feixe eletrônico, tornando essas fontes não adequada para o uso em microanálise, além de se tornarem pouco empregadas devido a necessidade de elevado vácuo (MALISKA, 2022).

Uma das técnicas que se tornou amplamente explorada em diferentes áreas é o sistema de espectroscopia de energia dispersiva de raios-X (EDS), o qual pode ser acoplado ao MEV e permite observar a região da amostra estudada. Ainda, é possível saber a composição da superfície das partículas a serem analisadas. Além disso, a EDS é mais rápida na quantificação dos elementos. Para amostras desconhecidas é inicialmente feita a identificação dos elementos, para posterior quantificação. Nessa técnica, as cargas elétricas são produzidas através da conversão de energia dos raios X identificadas pelo detector de estado sólido que coletam cargas negativas e positivas. Assim, as cargas elétricas geram sinais que quando são processadas identificam essa energia e, por

consequência, identificam também os elementos que compõem a amostra (COSTA, 2016).

Assim, para qualificar e/ou quantificar essas substâncias ilícitas, são desenvolvidos métodos analíticos com seletividade e sensibilidade adequados. Inicialmente é realizado os testes de *screening*, os quais são testes preliminares, mais simples e de baixo custo que apenas verificam a presença dessas substâncias de interesse. Como geralmente são testes colorimétricos, podem acusar o "falso positivo" e por não serem específicos, se faz necessário outros testes para a confirmação da identificação (CARLIN; DEAN, 2013; GOMES, 2013). Após são realizados os testes ditos confirmatórios, estes são mais complexos e envolvem técnicas mais sofisticadas como as cromatografias, a espectrometria de massas e a ressonância magnética nuclear. Esses testes servem tanto para confirmar a presença da substância de interesse quanto para quantifica-la (CARLIN; DEAN, 2013).

Com isso, é de suma importância que ocorra o desenvolvimento de técnicas analíticas instrumentais para que assim haja uma diminuição nos possíveis erros na área forense, onde uma determinação inadequada pode criminalizar um inocente e/ou vice-versa. Além de a adaptação e o desenvolvimento de novas metodologias se faz necessário para suprir a demanda legal de identificação e estudo de novas drogas. Um exemplo disso é o trabalho realizado por Accioni et al. (2018) que avaliou anfetamínicos em duas matrizes biológicas distintas, cabelo e fluído oral, através de um LC-MS/MS. Há também os autores Wiedfeld, Skopp e Musshoff (2021) que avaliaram 156 analitos, dentre eles alguns anfetamínicos, em cabelo por espectrômetro de massa Q-TRAP. Além desses, há a autora, já citada anteriormente, que utilizou a cromatografia a líquido para avaliar anfetaminas em amostras de urina de motoristas de caminhão, Takitane (2013).

RESULTADOS E DISCUSSÃO

Este está dividido em dois capítulos, os quais se referem a dois artigos que foram desenvolvidos no Laboratório de Biotecnologia de Produtos Naturais e Sintéticos (LBIOP) e Central Analítica da Universidade de Caxias do Sul.

Capítulo I.

Recent advances in amphetamine analysis in alternative biological matrices – oral fluid and hair, by LC-MS/MS: a review

Submetido a revista Forensic Chemistry (Editora Elsevier)

Capítulo II.

Rebites: Determination of non-target compounds by HRMS and quantification of caffeine by NMR and in drugs used by professional drivers in Brazil

A ser submetido a revista Forensic Chemistry (Editora Elsevier)

CAPÍTULO I

Recent advances in amphetamine analysis in alternative biological matrices – oral fluid and hair, by LC-MS/MS: a review

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ABSTRACT

Amphetamines and their derivatives are central nervous system stimulants and agonists of major neurotransmitters, thereby causing effects such as hyperthermia, paranoia, and mainly inhibition of appetite. The use of these compounds has been recorded since 1910, but in the 1960s, the potential for abuse of these substances was determinate. Although amphetamines have been prohibited compounds since the 1970s, 34 million regular users of these substances were registered worldwide in 2020. Thus, the development of methods capable of assisting the identification of these compounds in analyses of alternative biological matrices has been necessary. In these, different characteristics of the matrices are regularly sought, such as: a less invasive collection manner, longer stability of the samples, and easier extraction, as well as longer periods of use of the compounds. In this way, this review aims to identify the latest advances in amphetamine and derivatives analysis using liquid chromatography mass spectrometry (LC-MS) on alternative biological matrices, such as hair and oral fluid. In this sense, this paper compiles information from 20 articles, among which 7 report information on analysis in oral fluids and 14 on hair analysis, that were published between 2012 and 2022 and provide information on the stability of compounds and extraction methods. Summarising, this review is a guide that indicates the main advantages and disadvantages of these alternative matrices to researchers interested in analytical methods for the identification of amphetamines.

Keywords: hair; oral fluid; phenylethylamine.

Acronyms and abbreviations: 3,4-methylened dioxinetylamphetamine (MDE); 5-(p-methylphenyl)-5-phenyl-hyanthoin (MPPH); Trichloroacetic acid (TCA); Trifluoroacetic Acid (TFA); Brazilian National Health Surveillance Agency (ANVISA); Amphetamine (AMPH); Benzylcgonin (BZE); Cannabidiol (CBD); Cannabinol (CBN); Ketamine (K); Cocaethylene (CT); Cocaine (COC); Codeine (COD); Coefficients of variation (CVs); Liquid chromatography (LC); Liquid chromatography coupled to mass spectrometer (LC-MS); Ultra-efficiency liquid chromatography (UHPLC–MS/MS); delta-9-tetrahydrocannabinol (THC); Desidroncetamine (DHNK); Design of experiment (DoE); Dried oral fluid spots (DOFS); Electrospray (ESI); Anhydrous methyl ester (AEME); Extraction with weak ion exchange tips (WAX-S); Solid Phase Extraction (SPE); Oral Fluid (FO); Methamphetamine (MA); Methyldietanolamine (MDEA); Methylenedoxyamphetamine (MDA); Methylenedioxymethamphetamine (MDMA); Monoacetylmorphine (6-MAM); Morphine (MOR); Norketamine (NK); Norcissism (NCOC); New psychoactive substances (NPS); Salting-out (SALLE); Scientific Working Group for Forensic Toxicology (SWGTOX)*;* Central nervous system (CNS); Peripheral nervous system (SNP); Society of Hair Testing (SoHT)*;* Supramolecular solvent with restricted access properties (SUPRAS-RAM).

1. INTRODUCTION

Amphetamine (AMPH) was discovered in 1910 and, initially, its use was free and intended for therapeutic treatments for diseases, such as hyperactivity and narcolepsy. During World War II, its use was intended to keep soldiers alert and less fatigued. It was only in the 1960s that it was determined to have high potential for abuse and addiction, and this compound was banned in the United States of America in the 1970s. Despite this, 34 million users of AMPH were registered worldwide in 2020, making it the third most consumed class of drugs in the world [1–3]. Amphetamine and its mains analogues are synthetic substances that have a replaced phenylethylamine structure and stimulate both the central (CNS) and peripheral (SNP) nervous systems (Fig. 1) [4].

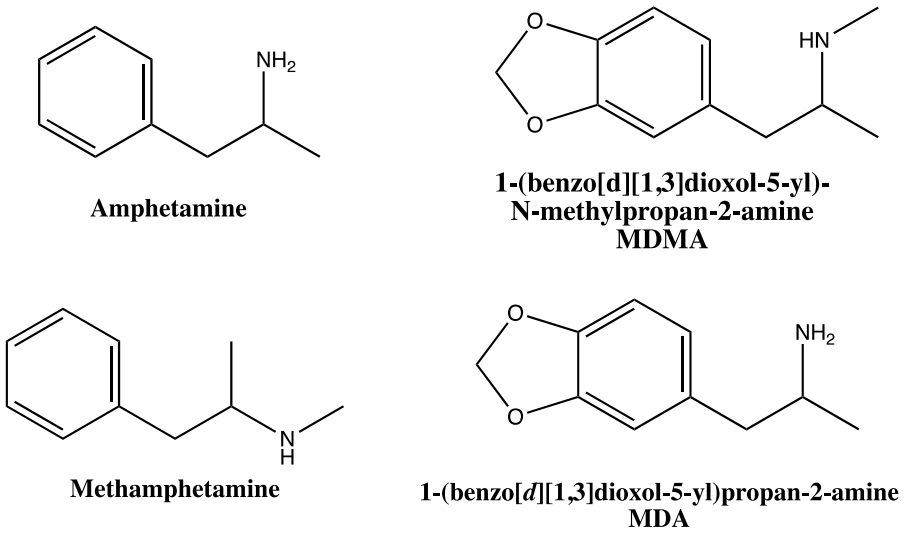

Fig. 1. Chemical structures of amphetamine and its derivatives.

AMPHs are commonly administered orally, acting as an agonist of the main neurotransmitters (dopamine, serotonin, norepinephrine, and noradrenaline) by inhibiting their degradations and recaptures, causing irreversible lesions in nerve cells due to their excess in the synaptic cleft. This action in the CNS causes effects such as increased self-confidence, increased socialisation, euphoria, and alertness. In addition to these, there are some adverse effects, such as hyperthermia, appetite inhibition, paranoia, and hyperactivity [3,4].

Although these compounds are controlled, new analogues appear every day, which are based on small structural alterations in the active molecules, obstructing the work of the organs responsible for the control of illicit drugs. Thus, innovations are needed in the development of

methods for analysis of these compounds, using analytical tools capable of analysing non-target compounds that are subsequently added to the list of prohibited compounds and included in new analytical methodologies [2,3].

As important as the analytical procedure is the extraction method, enabling the use of less invasive matrices or the ability to provide more complete information about the time of the active substance in the body. In this sense, blood and urine are normally used for analysis of illicit compounds and are more common in commercial laboratory routines; however, using these matrices, most AMPHs are analysed based on their active metabolites, which are usually more polar with a half-life time in the organism of less than 5 days on average [2,3]. Finally, blood and urine can be more easily degraded, and adequate temperature and pH storage is required (Fig. 2).

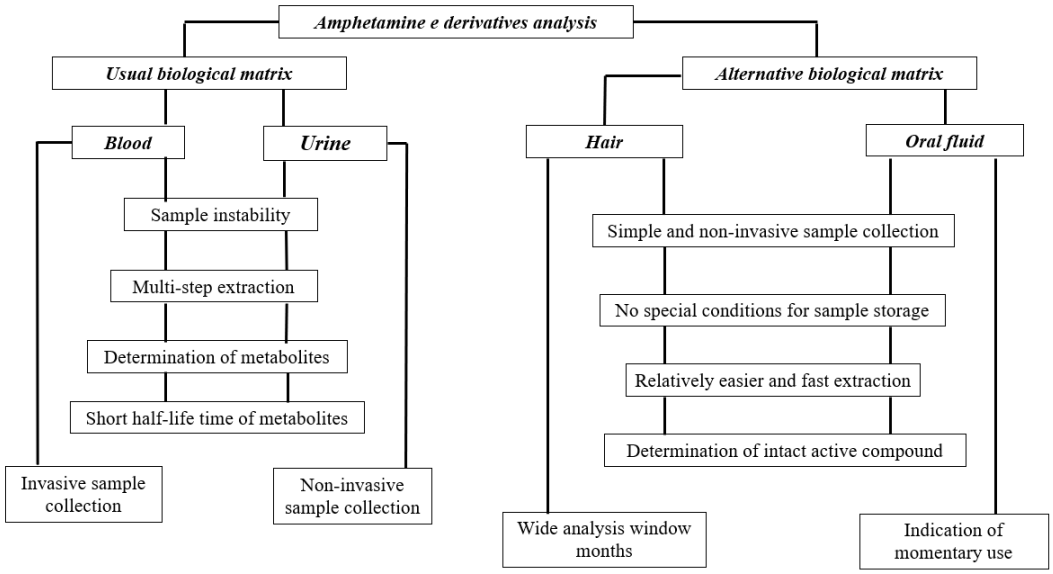

Fig. 2. Comparison between different matrices for amphetamine and derivatives analysis.

Looking for alternative matrices, researchers have sought the identification of these compounds in oral fluid and hair, which present some advantages in relation to the two mentioned above. It is possible that analysis of unchanged compounds in oral fluid or hair permits the enlargement of the compound's identification window. In a recent example, both advantages were observed by Accioni *et al.* [5], who evaluated MDA and MDMA in both matrices using LC-MS as an analytical method. To perform the analyses, the authors used an alkaline digestion and water-induced SUPRAS-RAM as extraction methods for hair and oral fluid samples, respectively.

Thus, this review aims to identify the main studies for the analysis of amphetamines and derivatives in alternative biological matrices, such as hair and oral fluid, through liquid chromatography coupled to mass spectrometry (LC-MS). In this sense, 20 articles were reviewed, and the pre-analytical and analytical methodology, as well as the validation of the methods, are discussed. The main details about the analysis methods are shown in the Table 1.

2. ALTERNATIVE BIOLOGICAL MATRICES

2.1. ABOUT ORAL FLUID

The use of oral fluid (OF) as a biological matrix has been highlighted in recent years in toxicological analyses because its collection is relatively simple (through swab and/or appropriate devices) and also non-invasive. OF is a mixture of saliva (secretion of three main glands), water, enzymes, glycoproteins, and electrolytes. The volume produced in a healthy individual is about 500 to 1,500 mL per day at a rate of 0.5 mL min^{-1}, and its pH ranges from 5.6 to 7.9. Thus, drugs with basic characteristics, such as amphetamine and derivatives, are found in greater quantity in OF compared to blood [6].

Among other advantages is the simplicity of its collection method, which brings a transparent and non-invasive sampling scheme while respecting the privacy of the donor. After collection with a swab, the OF is placed directly in a sampling tube, eliminating the need for training skills for the sample collector. Furthermore, there is a reduction in the probability of adulteration because there is a visualisation from the moment of collection until the sample filler [7,8]; however, the main disadvantage is the possibility of contamination by drugs consumed orally and the difficulty in producing saliva [6].

In addition, the kinetics of illicit drugs is different in OF compared to other matrices, whose main mechanism of transfer of active compounds from the blood to the oral fluid occurs by passive diffusion. This partition ratio can be affected by several parameters, such as composition and pKa, as well as the lipophilicity of compounds, which end up influencing the detection of the drug in OF. Drug-protein and OF-pH interactions are also factors that affect drug detection; this is because the binding of drugs to plasma proteins varies from substance to substance and also depends on individual susceptibility [9,10].

From this, drugs considered basic, with low molecular weight and a low rate of binding to plasma proteins, tend to accumulate in OF due to the lower pH than in the plasma, which promotes ionisation and prevents their return to the bloodstream. Still, only precursor drugs,

not metabolites, are usually found in OF because they are more liposoluble and, therefore, pass more easily through the membranes of capillary cells and acinar to the OF [9,10]. For example, THC is detectable in OF only for a few hours after consumption, in contrast with alkaline drugs, such as amphetamines, which could be found in higher concentrations in OF in comparison with the blood. However, a significant limitation of OF is related to its preservation and, in some cases, dilution with tampons. The traditional method of collecting oral fluid samples requires low-temperature storage from the collection site to arrival at the test facilities, where they are usually frozen if not immediately analysed [7,8].

2.2. ABOUT HAIR

Hair strands have a complex structure composed of several layers and specific structures. In this way, it can be separated into two main structures, which are the hair follicle and hair. In the first is the hair bulb, which is responsible for hair growth, while in the second, we have the visible part on the body surface [4]. The hair grows in cycles, alternating between growth and quiescence phases. The first phase is anagen, which is characterised by intense cellular activity of the cells of the matrix. After that, there is the phase called catagen, a transition phase where the interruption of cell division in the bulb occurs, in which it becomes completely keratinised and starts the degradation process. Then, there is the telogen phase, which is a period of quiescence where the hair stops growing completely and the deterioration of the follicle occurs and, consequently, the fall of the strand. Finally, after a certain period, the bulb re-establishes itself and the cycle resumes [4]. Thus, the recommendation of the Society of Hair Testing (SoHT) is to use an average growth rate of 1.0 cm $month^{-1}$ for calculations involving hair collection in the head region [4].

Henderson [11] suggests that the incorporation of substances into the hair occurs by three main routes. The first is by passive diffusion of substances to follicle matrix cells through the blood network, which is the most studied and proven pathway through experiments with mice. In this way, drugs and their metabolites penetrate the hair by passive diffusion to the cells of the growing matrix at the base of the hair follicle through the blood capillaries. Thus, as the hair grows, the drug would be incorporated into the matrix, and this incorporation would be dependent on the concentration of the drugs present in the bloodstream and, consequently, on the dose ingested. However, weak correlations were observed between the dose ingested and the concentration in the hair [4].

The second route is by spreading from sweat and sebum to the already formed wires, where there is the excretion of these drugs. Usually, the concentrations in sweat are higher than in the blood. In this way, these drugs could be transported along the length of the strand, where they would be incorporated by the hair. In the sebum, the incorporation of drugs would take place in newly formed strands that have not yet reached the surface of the skin [4]. Finally, the third route is through contamination from the external environment, which can occur through exposure in a contaminated environment, either by being close to the user of vaporised drugs or even by direct manipulation of drugs. Thus, the SoHT recommends washing samples in several stages with organic solvents and aqueous solutions [4].

With that in mind, the main advantage of using hair as an alternative biological matrix is the wide detection window of drugs of abuse due to its possibility of presenting prolonged drug exposure. However, exposure to natural factors is its main disadvantage, which can lead to contamination of the matrix and a reduced concentration of the drug present in it [6].

3. ANALYSIS OF AMPHETAMINES AND DERIVATIVES IN ORAL FLUID AND HAIR

Despite being banned drugs since the 1970s, the number of users of amphetamines and derivatives has had a recurrent exponential growth. Since they are of relatively easy chemical synthesis, it is a class of drugs that has been cleverly modified over the last few years, giving rise to compounds known as cathinones [12]. Thus, the need for new analytical methods for the analysis of these compounds is recurrent, including the use of hair and oral fluids as matrices. The Table 1 summarises the main characteristics of the articles published between 2012 and 2022.

Table. Summary of papers and their characteristics.

Reference	Analyzed compounds	Biological matrix	Equipment	Column	Mobile phases	Analysis time
Strano-Rossi *et al.* [15]	Benzylpiperazine, methylone, 5,6-methylenedioxy-2-aminoindan (MDAI), fenproporex, 4-fluoroamphetamine (4-FA), 4-methyl-N-ethylcathinone (4-MEC), 4-methylamphetamine (4-MA), methylbenzodioxolylbutanamine (MBDB), mephedrone, methylthioamphetamine (MTA), methylenedioxypyrovalerone (MDPV), mefenorex, nabilone, furfenorex, clobenzorex, JWH-200, AM 694, JWH-250, JWH-073, JWH-073, JWH-073, JWH- 073, JWH-073, JWH-250 122, HU 210 and CP 47497	Oral fluid	Agilent 1290 Infinity and an Agilent 6460 Triple Quadrupole Mass Spectrometer with Jet-Stream Electrospray Ionization Source	Kinetex C18 (2.6 μm, 100 mm × 2.1 mm)	5 mM ammonium formate with 0.05% formic acid (A) and methanol:acetonitrile (1:1) with 0.1% formic acid (B)	10 min
Chang *et al.* [16]	MA, AMPH, MDMA, MDA, K, norketamine, dehydronorketamine, 6-AM, MOR and COD	Hair	HPLC type Agilent 1100 LC coupled to a triple-quadrupole	Kinetex C18 (50 mm × 4.6	deionized water with 0.1% formic acid (A)	8 min

			mass spectrometer type Sciex API 3000 equipped with a Turbo IonSpray interface	mm × 2.6 µm)	and methanol with 0.1% formic acid (B)	
Montesano, Johansen and Nielsen [17]	96 drugs from different groups: opiates, amphetamines, hallucinogens, benzodiazepines, antihistamines, antidepressants, antipsychotics, barbiturates and other sedatives and muscle relaxants	Hair	ACQUITY UPLC and a Waters® TQ spectrometer	Acquity UPLC HSS C18 (150 mm × 2.1 mm, 1.8 µm)	5 m of ammonium formate at pH 3.0 (A) and 0.1% formic acid in acetonitrile (B)	19.5 min
Choi *et al.* [18]	MA, AM, MDMA, MDA, phentermine, fenfluramine, norfenfluramine, phendimetrazine, phenmetrazine, amfepramone, diethylnorephedrine, K and norketamine	Hair	Agilent 1200 LC coupled to an Applied Biosystems Sciex API 3200 Qtrap MS/MS MDS	Scherzo SS-C18 (3 × 150 mm, 3 µm).	35 mM acetic acid/90% acetonitrile in water (A) and 10 mM ammonium acetate/90% acetonitrile in water (B)	30 min
Paulo [4]	BZE, (±)-AMPH, (±)-MA, (±)-MDA, (±)-MDMA e (±)-MDEA	Hair	1290 Infinity Ultra Performance Liquid Chromatograph	Poroshell 120 EC-C18 (50 mm × 4.6	ultra-pure water with 0.1% formic acid (A)	4 min

			(CLUE) coupled with a triple-quadrupole mass spectrometer and an electrospray ionization (ESI) source	mm × 2.7 μm)	and methanol with 0.1% formic acid (B)	
Accioni *et al.* [5]	AMPH, MA, MDA, MDEA e MDMA	Hair and Oral fluid	Agilent Technologies LC 1200 coupled with a 6420 triple quadrupole mass spectrometer	Kromasil C18 column (4.6 × 150 mm i.d., particle size 5 μm)	0.1% aqueous formic acid solution (A) and a 0.1% methanolic formic acid solution (B)	15 min
Reinstadler *et al.* [19]	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, 3,4-methylenedioxyphetamine, 3,4-methylenedioxymethamphetamine, 6-acetylmorphine, AMPH, BZE, buprenorphine, COC, COD, methadone, dihydrocodeine, MA, and norbuprenorphine	Oral fluid	Eksigent 425 LC and TripleTOF 5600+ (both Sciex)	HALO Phenyl Hexyl (150 × 0.5 mm, 2.7 μm)	methanol in 0.5% aqueous acetic acid solution	10 min

Fabresse *et al.* [8]	Morphine, 6-MAM, COD, COC, BZE, AMPH, MA, MDA, MDMA, MDEA, THC	Oral fluid	UHPLC type Dionex Ultimate 3000, Thermo, TSQ Endura Thermo mass spectrometer	Hypersil GOLD Thermo (100 × 2.1 mm, 1.9 μm)	2 mM ammonium formate with 0.1% formic acid (A) and methanol (B)	8 min
Zhuo *et al*. [20]	MA, AMPH, MDMA, MDA, MDE, MOR, (6-MAM), COD, K , NK, COC e BZE	Hair	UPLC Waters Acquity system coupled to an API 4000QTRAP mass spectrometer	Restek Allure PFP propyl column (100 9 2.1 mm, 5 μm) with an Agilent Zorbax C8 pre-column (12.5 × 2.1 mm, 5 μm)	20 mmol/L ammonium acetate buffer with 0.1% formic acid (A) and acetonitrile (B)	10 min
Aijala, Wu and DeCaprio [21]	AMPH	Hair	Agilent 6470 Triple Quadrupole	Agilent Technologies 1.8 μm Zorbax Eclipse Plus C18 (2.1 ×	water with 0.1% formic acid and 5mM ammonium formate (A) and methanol with 0.1% formic acid (B)	8 min

				150 mm; 1.8 μm)		
Bassoti *et al.* [22]	COC, BZE, CT, D-9-THC, buprenorphine, 6-MAM, MOR, COD, methadone, EDDP, AMPH, MA, MDMA, MDA, MDE, K, MBDB	Oral fluid	Sciex 4500 triple quadrupole coupled to a UHPLC	Hypersil PFP Gold (50 × 2.1 mm, 1.9 mm)	Ultrapure water with 0.1% formic acid (A) and methanol:acetonitrile (50:50) with 0.1% formic acid (B)	12 min
Cooman *et al.* [23]	mCPP, JWH-018, JWH-073, XLR-11, AB-FUBINACA, AB-PINACA, MAM-2201, mCPP, MMB-FUBINACA, AM-2233, PV8, α-PVP, JWH-081, PB-22, 3,4-MDPV, JWH-122, AM-2201, AB-CHMINACA, UR-144, AB-PINACA, AB-FUBINACA, 3.4-MDPV, AM-2201, XLR11, MAM-2201, JWH-018, NPB-22, SDB-006, FUB-PB-22, THJ2201, MDMB-CHMCZCA, MN-18 and α	Oral fluid	Agilent 1290 Infinity LC coupled to an Agilent 6470 Triple Quadrupole MS/MS	Agilent Zorbax Eclipse Plus C18 (3.0 mm × 50 mm, 1.8 μm)	0.1% formic acid in 5 mM ammonium formate (A) and 0.1% formic acid in methanol (B)	12 min

	-PVP					
Chen *et al*. [24]	MA, AMPH, MOR, 6-MAM, K, norketamine, MDMA and MDA	Hair	ACQUITY UPLC coupled to a XevoXevo TQ-S Micro Triple Quadrupole Mass Spectrometer	UPLC BEH C18 (2,1 mm × 50 mm, 1,7 μm)	acetonitrile (A) and 10 mmol/L ammonium acetate with 0.05% ammonium hydroxide (B)	4 min
Gorziza *et al.* [9]	AMPH, MA, K, BZE and mitragynine	Oral fluid	Agilent Technologies Liquid Chromatography 1290 Infinity II with ESI(+) Ionization	Zorbax RRHD C18 (3.0 × 50 mm, 1.8 μm)	0.1% formic acid and 5 mM ammonium formate in water (A) and Acetonitrile with 0.1% formic acid (B)	10 min
Mannocchi *et al*. [25]	36 synthetic cannabinoids, 22 fentanyl analogues and/or their metabolites, 16 synthetic cathinones, 7 tryptamines and 6 phenethylamines	Hair	UPLC Acquity I Class coupled to a Waters XEVO TQ-S Micro tandem quadrupole mass spectrometer	Oasis HLB (5 μm 4.6 × 20 mm)	12.5 mM ammonium formate pH 9.5 (A) and acetonitrile (B)	10 min
Müller *et al*. [26]	Fenproporex, mazindol and amfepramone, AMPH, MA, methylenedioxyamphetamine,	Hair	Acquity® I-Class UPLC coupled to a Waters technologies	Acquity® UPLC BEH C18 (2.1 ×	water with 0.1% formic acid (A) and	2.2 min

	methylenedioxymethamphetamine, COC and metabolites, COD, MOR, 6-acetylmorphine and tetrahydrocannabinol		Xevo® TQS-micro triple quadrupole mass spectrometer	50 mm, 1.7 µm)	acetonitrile with 0.1% formic acid (B)	
Shin *et al*. [27]	three phytocannabinoids (CBD, CBN and THC) and two THC metabolites (THC-COOH and THC-COOH-glu), as well as six AMPH (AMPH, MA, phentermine, MDA, MDMA and MDEA)	Hair	Nexera II UHPLC type chromatograph coupled to a Q Exactive mass spectrometer	ZORBAX Eclipse Plus C18 (100 × 2.1 mm, 1.8 µm)	0.1% formic acid in water (A) and 0.1% formic acid in methanol (B)	13 min
Wiedfeld, Skopp and Musshoff [28]	156 analytes, including: benperidol, chlorprothixene, etomidate, flupirtine, fluvoxamine, medazepam, opipramol, pipamperon, reboxetine, tiapride, zotepine and zuclopenthixol, clothiapine, ethylphenidate, naltrexone, norclobazam, noscapine, primidone and viloxazine	Hair	Agilent 1290 Infinity LC and an AB SCIEX QTRAP 6500 mass spectrometer	Bifenil Kinetex (100 Å, 50 × 2.1 mm, 2.6 µm)	5 mM ammonium formate in 0.1% formic acid (A) and 5 mM ammonium formate in 0.01% formic acid in methanol (B)	8 min
Chen, Lee and Chang [29]	heroin (MOR, 6-AM), AMPH-type stimulants (AMPH, MA, MDA, MDMA), and ketamine (K, NK)	Hair	Nexera X2 HPLC with LCMS-8060	Kinetex C18 (50 × 2.1 mm, 2.6 µm)	water with 0.5%	7 min

			triple quadrupole mass spectrometer equipped with electrospray ionization (ESI), both Shimadzu, Kyoto, Japan		formic acid (A) and ACN with 0.5% formic acid (B)	
Gürler *et al.* [28]	benzoylecgonine (BEG), morphine (MOR), codeine, 6-monoacetylmorphine (6-MAM), heroin, tetrahydrocannabinol (THC), amphetamine (AMPH), 3,4-methylenedioxyamphetamine (MDA), buprenorphine (BUP), methamphetamine (mAMP), 3,4-methylenedioxy-Nmethylamphetamine (MDMA), and cocaine (COC)	Hair	Shimadzu LCMS-8030-plus (UFLC XR model LC-MS/MS, ESI technology).	Shim-Pack Column (FC-ODS 150 mm × 2.0 mmID, 3 μm, Shimadzu)	10 mM ammonium format (A) and 100% methanol (B)	4 min

3.1. AMPHETAMINES ANALYSIS IN ORAL FLUID

For analysis of amphetamines, THC, and derivatives in drivers, Strano-Rossi *et al.* [13] collected OF samples with a DCD5000 (Draeger, Germany) device, which allows the collection of about 0.3 mL of sample by swab. After, the compounds were extracted by centrifugation of the swab at 4,000 rpm for 5 min, and the resulting sample was stored at -20 °C. Then, 250 µL of each sample were diluted with 250 µL of mobile phase A, and 25 µL of the mix of internal deuterated standards, which were AMPH-D_5, MA-D_5, and THC-D_3, at 1 µg/mL were added. As a result, the authors were able to evaluate 24 substances covering different classes in a total run of 10 min, and from this, they obtained a detection limit of 1 ng/mL for most of the drugs evaluated. The method was applied to 400 real samples, and only one case of benzylpiperazine at 150 ng/mL, another of MDPV at 2 ng/mL, and one of 4-MEC at 200 ng/mL were identified. The method developed has the absence of a pre-treatment in the sample as a main advantage, making the analysis process faster, which is an important factor for routine analysis. Another advantage of the method developed by the authors is the use of Jet-Stream technology that optimises ESI conditions through the improvement of desolvation and the spatial focus of ions, which requires a smaller amount of sample. Furthermore, this technology increases the sensitivity and yield of the sample by improving the robustness of the assay and reducing the limits of detection and quantification of the method.

On the other hand, Accioni *et al.* [5] have directly extracted oral fluid, blood, urine, and three other biological matrices through a supramolecular solvent with restricted access properties (SUPRAS-RAM), which was produced in situ in the samples. For OF, 900 µL of the samples were basified with ammonia hydroxide 25% at 0.1 M, and 900 µL of tetrahydrofuran and 200 µL of 1-hexanol were added. For analysis, the authors used MA-D_{14} as an internal standard. In the results, the authors report a decrease in the matrix effect, with recovery between 75% and 100% of the analysed compounds. The low matrix effect is due to the use of SUPRAS-RAM, which is a great alternative since polysaccharides and proteins are excluded by size and precipitation, respectively. Furthermore, the authors obtained a universal platform for the treatment of samples with SUPRAS-RAM, since they used several biological matrices.

Reinstadler *et al.* [17] performed OF extraction using the aid of solid phase extraction Strata-X cartridges (Phenomenex, Torrance, USA). The samples were previously prepared as follows: 500 µL of OF was packed in the SPE cartridge after the internal standards (10 µL buprenorphine-D_4, 1000 ng/mL norbuprenorphine-D_3, and 500 ng/mL of the others IS's) were

added In this work, the analysis of undirected LC-MS/MS can comprehensively detect and identify drug compounds in OF with no restrictions, by relating the chemical properties of the compounds with the technique for automated data acquisition and the availability of reference spectra. The compounds were detected with efficiency and limits in the ng/mL range for molecules with logP ranging from 0.5 to 5.5. Furthermore, the authors also performed tests on a representative set of 59 real samples, and more identifications were obtained in comparison with a targeted workflowA comprehensive analysis, which was able to identify 524 compounds and their metabolites. The untargeted LC-MS/MS method was able to identify 34.4%, the conventional method identified 20.4%, and the combined method was able to identify 45.2% of compounds. Among the identified compounds were drugs such as COC, THC, MDMA, MA, MDA, and 6-acetylmorphine.

Fabresse *et al.* [7] used two extraction methods, one for THC and the other for the other drugs evaluated, but both used the solid phase extraction method and a Strata-Drug-X-B® cartridge (Phenomenex). For amphetamines, the authors used the following extraction method: the cartridge was conditioned with 1 mL of methanol and 1 mL of sodium acetate buffer (pH = 5). Then, the samples were eluted with 1 mL of 1% formic acid solution and 1.5 mL of sodium acetate buffer (pH = 5), agitated in vortex, and centrifuged at 4 °C for 10 min. The method was also applied to 127 real samples, where the analyses of interest were detected at least once, except methamphetamine. Amphetamines were also less detected, which can be explained by prevalence data, since amphetamines are less consumed than cannabis and cocaine, for example. Contamination of the oral mucosa with THC or cocaine after consumption facilitates its detection, but this is not the case of opiates or amphetamines since they are excreted only by saliva and therefore require a larger volume of sample. Thus, this variability of the sample volume is the main disadvantage of this method, which requires the use of a storage buffer to ensure sample stability, since analyses are usually not performed within 24 hours of collection.

Bassotti *et al.* [20] used the ‘dilute-and-shoot’ method, which consists of diluting 200 mL of OF samples with 200 mL of internal standards. In this method, 15 distinct drugs marked with stable isotopes, including cocaine, BZE, CT, D-9-THC, buprenorphine, 6-MAM, MOR, COD, methadone, EDDP, AMPH, MA, MDMA, MDA, MDE, ketamine, and MBDB, were tested. In pre-treatment, the OF was centrifuged for 10 min at 14,000 g-force, then the supernatant was transferred to vials and 2 uL were injected into the LC-MS/MS. The main advantage of the method is the non-use of purification methods, because despite bringing the desired sensitivity, the techniques are not available in all laboratories or the sampling strategies are very different. In addition, the authors used Quantisal® (Thermo Fisher, Waltham,

Massachusetts, USA), a reliable kit for the collection of OF, which in contrast with other kits, presents a volume indicator and a buffer solution.

On the other hand, Cooman *et al.* [21] performed sample preparation with SPE Bond Elut Certify cartridges (Agilent Technologies, Santa Clara, CA) and tested twenty-four synthetic cannabinoids and cathinones, which were quantified by LC-MS/MS. After sample collection, they were frozen at -20 ºC for further analysis. Furthermore, for elution of the analytes in the cartridge, 1 mL of 1-butanol:methylene chloride:acetic acid (24.5:75.0:0.5) and 1 mL of methanol:ammonium hydroxide (98:2) were used. The authors used the following compounds as internal standards: mCPP, JWH-018, JWH-073, XLR-11, AB-FUBINACA, AB-PINACA, MAM-2201, MMB-FUBINACA, AM-2233, PV8, α-PVP, JWH-081, PB-22, 3, 4-MDPV, JWH-122, AM-2201, AB-CHMINACA, UR-144, NPB-22, SDB-006, FUB-PB-22, THJ2201, MDMB-CHMCZCA, and MN-18. Cooman *et al.* [21] also evaluated 24 SPL in oral fluid, but different from the other authors mentioned, the samples were verified through a blind study where only two compounds, mCPP and MN-18, showed a general bias outside ±20%.

Gorziza *et al.* [8] performed only one extraction method with the aid of Whatman 903® filter paper in a weak ion exchange process (WAX-S). This consists of a solid phase extraction (SPE) and a salting-out assisted liquid-liquid extraction, i.e., the reduction of mutual miscibility between water and a water-miscible organic solvent through an electrolyte. The extraction procedure consists of 3 steps of aspiration and dispensing of the entire solution using the WAX-S tip. After this step, 100 µL of the top layer of the final solution were transferred to a glass bottle and analysed via LC-MS/MS. The authors used the following compounds as internal standards: AMPH, MA, ketamine, BZE, and mitragynine. This has advantages such as purity, consistency, uniform thickness, pH, and excellent absorption capacity, which favours drying times and storage options. In addition, the risk of contamination and exposure is lower, as only a few pathogens can survive on paper for long periods. Furthermore, the drying process of dried oral fluid spots (DOFS) increases the stability of the sample compared to liquid oral fluid; however, the recovery of the drug was not uniform for all compounds and was not completely satisfactory for mitragynine. Therefore, it may be an alternative to improve the recovery of the drug with the same amount of oral fluid, but this method also showed a smaller recovery for benzoylecgonine. Tips are a simplified version of SPE that require less load capacity and a simpler activation and elution process. Like Cooman *et al.* [21], the authors also conducted a study to evaluate the efficiency of the method using simulated samples, and all target compounds were correctly identified and quantified in all mixtures. Finally, the DOFS method

stands out as a more economical and easily accessible procedure, as well as the ‘dilute-and-shoot’ method of Bassotti *et al.* [20].

3.2. AMPHETAMINES ANALYSIS IN HAIR

The extraction of drugs in hair can be through acid, solvents, alkaline digestion, or enzymatic hydrolysis. In comparison, acid and with organic solvents is simpler and requires more time, while extraction by alkaline digestion or enzymatic hydrolysis is faster with destruction of the protein matrix. However, for chemically instable compounds, such as 6-AM (heroin marker), alkaline digestion is not adequate, while enzymatic hydrolysis has a high cost when compared to other methods. Both methods require more cleaning procedures to eliminate interferences in the analysis [14].

In this way, Chang *et al.* [14] have performed a microwave-assisted extraction method (MAE), which requires shorter extraction time, facilitates the rapid and selective desorption of complex matrix compounds like hair, and allows simultaneous extraction at high pressure and temperature; however, if the temperature is not suitable for extraction, the compounds may be degraded. The authors started the process by decontamination of the hair with 2 mL of DCM for 5 min. After drying at 40 °C, each sample was cut into pieces smaller than 1 mm and weighed about 10 mg individually. Then, 0.5 mL of trifluoroacetic methanol-acid (TFA) (8.5:1.5) and 200 pg mg^{-1} of each IS were added to each sample, including: MA, AMPH, MDMA, MDA, K, NK, desidronorcetamine (DHNK), 6-MAM, MOR, and COD. After the samples were placed in a microwave oven adjusted to 700 W for 3 min, the extraction solution was collected and 50 µL of methanol:HCl (99:1) was added. The solution was evaporated until dry at 40 °C under a nitrogen current, after adding 100 µL of deionised water:methanol (99:1), and 20 µL was injected directly into the LC-MS/MS. The MAE extraction method developed by the authors showed no obvious degradation of the compounds and their metabolites, with the exception of 6-MAM which is unstable. In addition, the authors evaluated 8 real samples, where 4 samples were positive for only one drug, two of which were positive for amphetamines. The other samples were positive for more than one drug concomitantly.

Montesano, Johansen, and Nielsen [15] analysed basic drugs, like amitriptyline, AMPH, bromazepam, buprenorphine, COC, dibenzepin, flunitrazepam, mianserin, MOR, and zolpidem, and acidic and neutral compounds, such as cyclobarbital, ibuprofen, paracetamol, and salicylic acid, simultaneously. To perform the tests, the authors segmented the hair samples

to an average size of 1 cm then weighed 10 mg of each sample, which were rinsed once with isopropanol and twice with water. After that, steel beads were dried up for further spraying. Then, 200 μL of extraction medium (ME) and 25 μL of internal standard were added for basic, acidic, and neutral drugs. Still, the hair was sprayed on a ball mill homogeniser for two cycles for 30 s at 6,500 rpm. The samples were centrifuged at 20 ºC for 3 min at 3,600 rpm and incubated in an oven at 37 °C for 18 h. After incubation, the tubes were centrifuged, the samples were diluted 1:1 with deionised water, and finally, 10 μL of the solution were injected in the equipment. The developed method allowed the detection of exposure to drugs in a single dose for some analyses through the low detection limit and the detection of chronic exposure to drugs through the linearity range. However, the quantification parameters only meet the acceptance criteria for 93 drugs out of the 96 tested, not including THC, CBN, and CBD. Unlike other authors, Montesano, Johansen, and Nielsen [15] performed analyses of several hair samples from children suspected of sexual abuse for a long period of time, where the target compounds are common medications in addition to sedatives.

Choi *et al.* [16] performed hair decontamination by washing with 2 mL of methanol, distilled water, and methanol in sequence. After drying, the samples were cut into pieces smaller than 1 mm, weighed to approximately 5 to 10 mg, and stirred in 2 mL of 1% HCl in methanol for 20 h at 38 °C. Before incubation, 50 μL of methamphetamine was added as an internal standard. The extract was evaporated until dry at 45 °C under gaseous nitrogen, and the residue was reconstituted in 200 μL of methanol. Finally, 5 μL were injected into the LC-MS/MS. The authors performed the preparation of the sample by ionising the amines in acid gradient so that they were strongly retained in the spine through ionic interactions with the anionic ligands of the column. After that, they neutralised the analyses during the elution of the pH gradient, providing good separation of low molecular weight amines due to their pKa and hydrophobicity values. As a result, the authors indicated that the standard reverse phase columns that are commonly used present difficulties in the separation of low molecular weight amines due to their structural similarity and present the same transition of monitoring of multiple reactions (MRM). Thus, the method developed is more suitable for low molecular weight amines, such as amphetamines, because it uses multimode reverse phase columns and a highly organic mobile phase, resulting in increased sensitivity of the analysis and enabling the detection of amines at trace level.

For Paulo [4], the extraction sample was 25 mg of a hair sample with length between 1.0 and 4.0 cm, more than twice as much as the previous authors. The procedure performed in triplicate uses 2 mL of dichloromethane (DCM) and BZE, AMPH, MA, MDA, MDMA, and

MDEA as standards. The author evaluated 14 real hair samples without any positive results to amphetamines, even in a sample where the donor indicated to be a user of ecstasy. According to the author, this result is related to the time of abstinence able to eliminate all traces of the drug. Despite this, the author detected benzoylecgonine, a metabolite of cocaine, in 77% of the samples. In three samples, the donors claimed to have gone at least 2 months without using crack or cocaine, so it is possible that the drug persisted in the body, especially in one of the donors where a sample of arm hair was collected. This sample presented an significant amount of benzoylecgonine, and it is believed that the user used the substance at a time when the hairs were developing, explaining the high concentration of the drug in the sample.

Still, Zhuo *et al.* [18] used ceramic beads to perform the extraction. After washing and drying, the samples were finely cut into 2–3 mm pieces. Then, 50 mg of hair were added to ceramic beads of 1 mm, along with 1 mL of methanol and a solution of 10 ng/mL of internal standards, including: MA, AMPH, MDMA, MDA, MDE, MOR, 6-AM, COD, K, NK, COC, and BZE. Then, the samples were homogenised in an OMNI Bead Ruptor 24 coupled to an OMNI BR-Cryo cooling unit (OMNI International IM, GA). After the evaluation of 158 real samples, 26 samples were positive for more than one class of drugs of abuse, limiting false-negative results; this is due to the multitarget method used by the authors. Amphetamines were also the most frequently found in about 142 samples, followed by opiates (n = 18), ketamine (n = 19), and cocaine (n = 12).

On the other hand, Aijala, Wu, and DeCaprio [19] developed a method from the fortification of the hair sample with amphetamine standards at pH 6. Subsequently, the sample was extracted with a mixture of methanol, acetonitrile, and ammonium formate 2 mM (25:25:50) at 37 °C. In another extraction, the previous steps were repeated, the enzyme proteinase K was added for extraction, and the samples were incubated for 24 h. Finally, in both cases, the samples extracted from both methods were centrifuged at 10,000 g-force using a 3 kDa molecular weight cutting PTFE rotary filter to separate the hair particles from the solvent mixture containing the compounds to be analysed. As a result, the authors obtained a higher average extraction through enzymatic hydrolysis, which resulted in 1.27 pg/mg, while the solvent extraction technique resulted in an average extraction of 608 pg/mg. Furthermore, variance was evaluated for both techniques, ranging from 110 to 2709 pg/mg and 0.0 to 6.51 pg/mg for enzymatic and solvent extraction, respectively. Based on the results, the authors indicate that the solvent extraction presents a smaller recovery compared to the enzymatic process, as well as an extended processing time.

Chen *et al.* [22] used MA, AMPH, MOR, 6-MAM, ketamine, NK, MDMA, MDA, and methoxyphenamine as internal standards. About 10 mg of hair was weighed for the samples, after being washed with ultrapure water and acetone. Then, the sample was dried, cut, and ground then 0.3 mL of methanol was added. Afterward, the sample was subjected to an ultrasonic water bath for 1 h at 4 °C and subsequently centrifuged at 13,000 rpm for 10 min. Finally, 100 µL of the supernatant were transferred and 1 µL was used for UPLC-MS/MS analysis. The method developed by the authors with methanol as a solvent and ultrasonic bath was able to extract the compounds through a simple dissolution with a shorter pre-treatment time compared to previous methods. As a result, in the real samples, the authors were able to determinate the presence of methamphetamine and amphetamine concomitantly.

Mannocchi *et al.* [23] used about 25 mg of hair, washed twice with dichloromethane, that was added to an internal standard solution containing 119 distinct compounds, including: AMPH, MA, MDA, MDMA, MDEA, MOR, COC, COD, and oxycodone. Then, the samples were cut and incubated with 500 µL of M3 reagent® (Comedical® s.r.l., Trento, Italy) at 100 °C for 60 min. Finally, the samples were cooled at room temperature, and 1 µL of the supernatant was injected. According to the authors, the use of the M3 reagent® reduced the pre-treatment time of the samples. A total of 10 real hair samples obtained from polyconsumers of classic drugs and unpublished SPL were also evaluated, in which the presence of at least three simultaneous substances were detected. In 7 samples, the presence of SPL of different chemical classes were identified, such as cathinone, tryptamine, synthetic cannabinoids, fentanyl analogues, and related metabolites; one sample contained 13 distinct substances, including MDMA. Another advantage of this method was the isomer determination, such as 5-APB and 6-APB.

Müller *et al.* [24] used stainless steel balls for the extraction of samples assisted by a ball mill. For this, the authors used about 20 mg of 3 to 4 cm long decontaminated hair and added 500 µL of methanol and 50 µL of the internal standard solution, which included: AMPH, MA, MDA, MDMA, amfepramone (AMFEP), fenproporex (FENP), mazindol (MZD), THC, COC, BZE, CT, NCOC, MOR, COD, 6-MAM, and anhydroecgonine methyl ester (AEME). Afterwards, the samples were pulverised in a ball mill (RETSCH 2000®) for 5 min at 30 Hz. Then, they were incubated for 15 h at 50 °C and 1,000 rpm. Finally, the spheres were removed with the aid of a magnet, and the samples were centrifuged again at 12,000 g-force for 10 min. The supernatant was transferred, and 1.5 µL was injected into the UPLC-MS/MS. The authors evaluated around 50 real samples of drivers involved in traffic accidents, obtaining positive results for COC (n = 3), MDMA (n = 2), and THC (n = 2).

Shin *et al.* [25] also employed stainless steel spheres for extraction, which began with about 10 mg of hair cut into approximately 1 mm sections. The samples were sprayed at 30 Hz for 20 min and 10 μL of the internal standard solution (including CBD, CBN, THC, THC-COOH, THC-COOH-glu, AMPH, MA, phentermine, MDA, MDMA, and MDEA) was added in addition to 1 mL of 0.5% formic acid in methanol. The extraction product was centrifuged at 13,000 rpm for 5 min, and about 800 μL of the supernatant was evaporated at 40 °C in N_2, after being treated twice with 0.5% formic acid in methanol. The authors evaluated 11 real samples; among them, 4 tested positive for the presence of amphetamines, such as AMPH, MA, MDA, MDMA, and phentermine, as well as the presence of THC, indicating the joint use of drugs.

Wiedfeld, Skopp, and Musshoff [26] used a total of 10 strands of hair in each sample, which were decontaminated by different methods. In one method, the strands were individually cleaned with a soft paper towel, while in the other, the sample was cleaned with the same paper towel soaked in methanol. For segmentation, the wires were fixed on a transparent tape strip (tesafilm® transparent tape, Tesa SE, Norderstedt, Germany) after being individually cut with the aid of a scalpel into average sizes of 2 mm. The samples are transferred directly to vials containing 30 μL of the methanol extraction solvent: 2 mM ammonium acetate buffer (25:75) with 5-(p-methylphenyl)-5-phenyl-hydritanin (MPPH) as an internal standard at a concentration of 16.6 ng/mL. Finally, the extraction was complete with the aid of a sonicator at 50 °C for 4 h. Despite the longer procedure, these authors have indicated the amounts of the compounds analysed in individualised hair strands; however, the sensitivity of the analytical method associated with individualised analysis limited the identification of benzodiazepines of the same drug class. For example, zolpidem and zopiclone were administered in the same dosage but only zolpidem could be detected.

Chen, Lee, and Chang [24] have previously decontaminated the samples with 0.5 mL of DCM for 3 min at room temperature then dried the samples in an oven at 45 ºC. A single strand of hair was cut at 0.4 mm near the end of the scalp. Then, 0.2 mL of methanol:TFA (85:15) and 200 pg/mg of internal standard were added. The hair samples were placed in the microwave at 700 W for 3 min then washed with 0.2 mL of methanol to ensure the dissolution of any drug that was in the hair. 50 μL of methanol:HCl (99:1) was added and evaporated to dryness at 40 ºC under a nitrogen flow. Furthermore, the authors performed the derivatisation of the sample to improve the detection characteristics of the compounds. Thus, the residue was dissolved in 100 μL of a sodium bicarbonate buffer (100 mM, pH 9.5 with NaOH). After, the organic layer was evaporated at 40 ºC under a nitrogen flow then the residue was reconstituted

with 40 μL of water:ACN (50:50) and injected into the LC-MS/MS for analysis. The authors only had a real sample to perform the analyses, which found MA and AMPH at concentrations of 1937.1 and 251.1 pg/mg, respectively.

In the same way, Gürler *et al.* [28] washed the samples with water, methanol, and acetone in triplicate. They were then cut to a length of approximately 1–2 mm, and 50 mg of the samples were weighed. After that, 2 mL of methanol were added, and the internal standard, being them: Nalorphine, THC-d3, AMPH-d5, MDA-d5, mAMP-d5, MDMA-d3. Then, 2 mL of 0.1 M HCl was added, and the sample was incubated for 3 h at 60 °C then centrifuged at 1780 g-force for 10 min. In the aqueous phase, 2 mL of 1 M NaOH was added, incubated at 90 °C for 45 min, and again centrifuged. The pH of the solution was neutralised then the solution was submitted to SPE extraction. The cartridge was conditioned with 1 mL of methanol and 1 mL of ultrapure water, the sample was loaded into the cartridge, and 1 mL of 5% methanol solution was passed. Then, the cartridge was left at room temperature for 20 min, and 1.5 mL of washing solution were passed (0.5 mL 100% methanol, methanol with 2% ammonia, and methanol with acetic acid of 2%). The authors did not test any real samples, indicating that access to these were blocked by the local laws of Turkey. Furthermore, the authors state that it is the first scientific validation study in the country with 12 compounds analysed simultaneously using LC-MS/MS.

4. METHODS COMPARATION

All studies present methods validated from different guides, with SoHT and SWGTOX being the most used, in addition to following authors such as Peters *et al.* [29]. From there, they used parameters, such as limits, precision, and selectivity, as well as linearity in ranges between 0.01 and 10,000 ng g^{-1}. As an example, Bassotti *et al.* [20] presented a linearity of an R^2 value of 0.99, evaluated from a curve of 6 points, while Cooman *et al.* [21] obtained an R^2 value of 0.98, with a linearity of 100 ng/mL. Montesano, Johansen, and Nielsen [15] also obtained an $R^2 > 0.99$ for all analytes, except for sertraline which obtained an $R^2 = 0.98$. For the other authors mentioned in this article, they obtained a $R^2 \geq 0.99$ for all compounds proposed in each study, with linearity from 0.2 to 250 ng/mL. Still, the cut-off values indicated by the cited authors ranged from 0.1 ng/mL to 300 ng/mL for oral fluid and 0.0076 ng/mg to 200 ng/mg for hair.

Regarding reproducibility, Bassotti *et al.* [22] indicated an intra-assay accuracy with variable variation coefficients (CVs) between 1.04% and 8.30%, while for inter-assay, CVs were between 3.37% and 10.14%. Müller *et al.* [24] obtained a general intra-assay CVs of 1.9% to 13.5%, while for inter-assay, the CV ranged from 3.3% to 14.3%. The highest percentages among the authors mentioned were by Chang *et al.* [14] and Choi *et al.* [16]. The first obtained <15% for intra-assay with six repetitions and inter-assay with three replicates for each analysis, while the second author reported <20% for low concentrations and <15% for high concentrations. Thus, for the lower levels, the accuracy obtained was from 9.5% to 10.1%, while for the average level, an accuracy of 9.8% to 13.6% was obtained. Cooman *et al.* [21] obtained an accuracy of 10.7% to 11.6%, while Gorziza *et al.* [8] also divided accuracy into three levels like Cooman *et al.* [21] but divided them into intra- and inter-assay as Bassotti *et al.* [20]. Thus, for the low level, an intra- and inter-day accuracy of 2 ng/mL was obtained. For the average level, an accuracy of 12 ng/mL was obtained for both amphetamine and methamphetamine. Finally, for the high level, the accuracy for amphetamines was 20 ng/mL. Zhuo *et al*. [18] obtained inter- and intra-assay precisions of less than 20%.

The Scientific Working Group for Forensic Toxicology (SWGTOX) [30] states that the suppression of analysing ionisation resulting from co-eluent compounds is a phenomenon found in LC-MS. Thus, the suppression should not exceed 25% or 15% of the CV, as it can impact the detection limit of a qualitative method and the limits of detection, quantification, and bias in quantitative methods. For Montesano, Johansen, and Nielsen [15], the suppression of ions was <20%. For Gorziza *et al.* [8], all compounds tested were within the acceptable range of <25%. For Cooman *et al.* [21], all target compounds and internal patterns showed suppression for low and high levels within the established range of ±20%, where the overall suppression was 6.4% for the low level and 3.9% for the high level. For Reinstadler *et al.* [17], 80% of the evaluated compounds had an identification limit ≤5.0 ng/mL. For Chang *et al.* [14], suppression was 3% to 19% for all compounds evaluated. According to Mannocchi *et al*. [23], ion suppression was 13% for all analyses evaluated. Shin *et al.* [25] found a slight suppression of ions from some analytes, which was compensated for by the use of internal dexterous patterns. Still, the matrix effect in the minimum values for amphetamines indicated by the evaluated authors ranged from -49.3%, indicated by the author Müller *et al*. [24], to 98%, indicated by the authors Chen, Lee, and Chang [27].

The evaluation of real samples is essential to identify the method application. In this line, Cooman *et al*. [21] identified 17 compounds of various concentrations that were higher than 20% of the reference, with the highest concentration of 50 ng/mL, while Fabresse *et al.*

[7] evaluated 127 samples, among which only 3 confirmed the presence of amphetamines. Strano-Rossi *et al.* [13] evaluated 400 OF samples and identified the presence of benzylpipezine at 150 ng/mL, MDPV at 2 ng/mL, and 4-MEC at 200 ng/mL in different samples. In these cases, the retention time did not differ more than 2.5% in relation to the standard, and all other evaluation criteria were met. In the same way, Mannocchi *et al*. [23] analysed 10 hair samples, which presented contemporary consumption of MDMA, while all users of the fentanyl analogue (n = 3) presented co-ingestion of amphetamine-type drugs and other classes. Also, these authors have detected mephedrone in a sample associated with low concentrations of MDMA and other drugs. Shin *et al*. [25] evaluated hair samples from 10 users of cannabinoids and/or amphetamines, of which four amphetamine users also tested positive for the presence of cannabinoids, indicating the simultaneous use of AMPH and cannabis.

The stability of the samples is important in forensic analysis, as it is necessary to store the counterproof for future analysis. Samples of Bassotti *et al.* [20] remained stable, as there was a variation below 15%, at a temperature of 20 ºC for 6 months and for a week at 4 ºC. The samples of Gorziza *et al.* [8] remained stable for 35 days at a temperature of 6 ºC. Meanwhile, the amphetamines evaluated by Fabresse *et al.* [7] remained more stable at 4 ºC for 7 days, while those with OF in Quantisal buffer® presented lower stability. Strano-Rossi *et al.* [13] have reported stability of the samples for two months after 8 freezing/defrosting cycles, in addition to 4-week stability at room temperature. Accioni *et al.* [5] obtained a stability for at least 3 days for OF samples and for up to 2 weeks for hair samples at ambient temperature and at 4°C. Shin *et al.* [25] presented the lowest stability in comparison; the samples were stored for 24 h at 15 °C. Müller *et al*. [24] obtained stability of all compounds for up to 15 h, but different from the other authors, the samples remained in the automatic sampler.

Cooman *et al.* [21] carried out an acceptable recovery for all internal standards. Bassotti *et al.* [20] obtained a recovery from 76.6% to 115.3% and found no matrix effects. Still, Gorziza *et al.* [8] obtained an average recovery of 82% for all compounds, as well as for amphetamines, in both extraction processes (DOFS and WAX-S), which is similar to results obtained for the recovery of dry blood stains. For the DOFS method of Gorziza *et al.* [8], an average recovery of 103% was obtained after the analysis of the target analytes during the 35 days maintained at 6 ºC. Shin *et al.* [25] obtained an average recovery of 85.9% to 100.0%, in addition to a matrix effect of 92.5% to 101.2% for amphetamines. Meanwhile, Chen *et al*. [22] obtained an average recovery above 72.9%, and the lowest percentage found among the authors mentioned in this article obtained a matrix effect between 92.7% and 109.2%. Choi *et al.* [16] achieved an average recovery of 83% to 104% and 95% to 105% at low and high concentrations, respectively.

Gorziza *et al.* [8], Fabresse *et al.* [7], and Montesano, Johansen, and Nielsen [15] were the only authors who evaluated the carryover parameter, which is defined by SWGTOX [30] as the appearance of unintentional signal in subsequent analyses which generate false positives. For Gorziza *et al.* [8], the experiments did not show the presence of carryover after injecting the highest concentration of the calibrator three times, followed by an evaluation of white. For Fabresse *et al.* [7] there was also no presence of carryover. In addition, the average transport was less than 20% of the LOD for all analyses and 5% for IS. Montesano, Johansen, and Nielsen [15] had a carryover of <0.1%.

In another way, Chen, Lee, and Chang [27] compared the traditional amount of hair commonly used and that used by them in order to investigate the difference in quantitative concentration of medications. From this, one of the differences was the amount of hair strands required for conventional analysis (more than 504 strands). With this, MA and AMPH concentrations of 1,937.1 and 251.1 pg/mg, respectively, were found. In the other samples, which only used one segment of the wire, the authors found methamphetamine and amphetamine in all samples, while MDMA and MDA were detected in sample 2 at 3,982.7 ± 1,180.2 pg/mg and 494.0 ± 142.0 pg/mg, respectively. From this, it is possible to indicate the importance of analysing segments of the hair in toxicological analyses because the number of samples available is often limited and usually only 1 cm or 10–20 mg of sample are used, which only measures for one month of drug intake. It is important to reduce the amount of hair and shorten the time from one month to a week, even for a day of drug intake.

5. CONCLUSION

In summary, this paper presents 7 articles for OF and 14 articles for hair published between 2012 and 2022 and indicates the main forms of extraction and analytical methods used for the identification of amphetamines and their derivatives. Some analyses also present other drugs of abuse that were evaluated together with amphetamines and their derivatives. In relation to extraction, the method developed by Bassotti *et al.* [20] for OF is more effective because the method is extremely simple and still presents excellent results in the sensitivity and quantification of compounds. In terms of running time, only 3 authors got times between 2 and 4 min; however, 9 authors raced between 8 and 10 min and only 2 authors performed analyses of more than 15 min. In terms of equipment used, 5 authors used equipment from the SCIEX brand and 3 authors used the same 1290 Infinity equipment of the Agilent brand. Finally, a C18

column was the most used, being adopted by 19 authors, this is due to the particles not being completely pore, leading to a faster mass transfer, resulting in less band expansion and achieving analysis of high separation efficiency. In addition, 14 authors use some concentration of formic acid in at least one of the mobile phases and 11 authors use methanol in at least one of their mobile phases. Thus, it is possible to affirm that all of the authors mentioned in this article presented robust methods because they evaluate a range of different drugs of abuse with validated methods and parameters within those required by international guidelines. With this, this article will serve as a guide for researchers and students who seek more information on analyses of these compounds in alternative biological matrices of hair and oral fluid.

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CAPÍTULO II

Rebites: Determination of non-target compounds by HRMS and quantification of caffeine by NMR and in drugs used by professional drivers in Brazil

A ser submetido a Forensic Chemistry

Rebites: Determination of non-target compounds by HRMS and quantification of caffeine by NMR and in drugs used by professional drivers in Brazil

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Abstract

Highways are fundamental to Brazil, which are the routes that carry about 75% of the country economy. Thus, professional drivers are induced to long daily journeys. Thus, there is a recurrent use of stimulates, many of which are sold without any control at gas stations, which may contain legal and illegal compounds. In this line, this work aims identify target and not-target compounds in "Rebites" by different analytical techniques. Thus, in the first stage by High Resolution Mass Spectrometry (HRMS), we tracked the compounds inserted in seven samples collected throughout the country, which indicated the presence of caffeine as the main active compound, in addition to compounds such as theophylline, lidocaine, chlorobenzorex, among others. others. In the second step, we tested the quantification of caffeine by qNMR and compared it with the results obtained by HPLC. Furthermore, the samples were evaluated by Scanning Electron Microscopy (SEM) with Energy Dispersive Spectroscopy (EDS). As results, this molecule was quantified ranging 14 and 31% (m/m) approximately. In summary, this study reports the chemical characterization of real samples of tablets obtained freely on Brazilian highways, being the caffeine is the main active, which had its content determined by qNMR, HPLC and SEM-EDS. The high amount of this compound, associated with other stimulating, depressors and hallucinogens indicates that Rebites should undergo appropriate quality control.

Palavras-chave: Rebites; Stimulants; Caffeine; qNMR, SEM-EDS.

Acronyms and abbreviations

CID	Collision-induced Dissociation
CNS	Central nervous system
EDS	Energy Dispersive Spectroscopy
ESI	Electrospray ionization
FEG	Field Emission Electron Guns
GC-MS	Gas chromatography coupled to mass spectrometry
HPLC	High-performance liquid chromatography
HRMS	High Resolution Mass Spectrometry
IS	Internal standard
LC-MS	Liquid chromatography coupled to mass spectrometry
LC-MS/MS	High-performance liquid chromatography tandem mass spectrometry
m/z	*mass/load*
MDA	3,4-methylenedioxyamphetamine
MDEA	Methyldiethanolamine
MDMA	3,4-methylenedioxymethamphetamine
NMR	Nuclear Magnetic Resonance
qNMR	Quanitative Nuclear Magnetic Ressonance
Q-TOF	Quadrupole-time of flight
SEM	Scanning Electron Microscopes
THC	Tetrahydrocannabinol
TOF	Time of flight

INTRODUCTION

The statistical report provided by the National Traffic Department of Brazil indicated that in 2021 the fleet of motor vehicles reached 111 million, where 192.4 thousand correspond to cargo transport automobiles [1]. In Brazil, highway transportation is the most used, so there is a wide road network, which increases consequently the probability of traffic accidents. until the month of September of the year 2021 have reached a total of 3 million traffic accidents [1,2]. In June 2022, more than 520,000 accidents had already been recorded, with more than 23,000 involving cargo transport vehicles [1]. Its main causes are related to speed, absence of the use of safety equipment and dangerous driving under the effect of psychoactive substances [3,4].

According to the Ministry of Infrastructure (*Ministério da Infraestrutura)*, truck drivers are involved in 4.41% of traffic accidents and approximately 10.0% of deaths caused by these accidents in Brazil. The use of psychoactive substances has a great impact on these numbers, as they increase the risk of fatal accidents five times compared to non-user drivers [1,5]. The economic pressure has caused a significant increase in the use of stimulants by professional drivers, who usually work for long hours [4,6].

Recently, a huge study has pointed the constant association between drivers and illegal drugs around the world [7]. The cocaine, amphetamine, tetrahydrocannabinol (THC) and opiates were found in samples of drivers from several countries such as: the United States, Brazil, Norway, Spain, Italy and Hungary, has been reported with divergent results between the types of drugs used and the world regions [7]. In a study recently, Yosef *et al.* [4] have reported that 70% of the 400 respondents of truck drivers in Ethiopia by had used psychoactive substances. In the same line, Zaharaddeen *et al.* [8] have indicated that 64.5% of the 152 of drivers interviewed in northwestern of Nigeria, making regularly use of stimulants, among which 40.8% and 26.5% justify with the desire to remain active or to decrease the effects of fatigue respectively. Also in Africa, a study conducted by Dada *et al.* [9], in southwestern Nigeria, showed that of the 306 interviewees, 67% reported using cough syrups for non-medicinal purposes, 49.3% reported misuse of analgesics and 33.0% reported amphetamine/methamphetamine use.

In this sense, in Brazil the pattern of use of these professionals are amphetamines and derivatives, other licit stimulants such as caffeine and illicit such as cocaine and derivatives have been reported. These come from commercial medicines, traditional drug trafficking as well as illegal drugs such as “rebites”, which can be found in most gas stations along Brazilian highways [10,11]. Stimulants act on the central nervous system and as a side effect cause euphoria, insomnia, increase the user's residence and combat fatigue. In contrast, they can cause paranoia and panic, tachycardia, increased blood pressure, and alter the psychomotor capacity of the driver [6,12,13] For analysis normally are used urine and blood samples, which are analyzed by LC-MS-MS or GC-MS. As instance Labat *et al*. [14] have reported the analysis of 1000 truck driver’s urine samples, being cocoine, amphetamines and canabidionol confirmed by GC-MS, meanwhile other drugs such as methadone, benzodiazepinics e buprenofina by LC-MS-MS. As results, 8.5% and 0.4% of samples have indicated the presence of cannabinoids and amphetamine respectively. In the same way, Dirago *et al*. [15] have reported the analysis of around 5000 blood samples by LC-MS-MS of drivers which have involvement in transit

accident. As result, 637 and 226 were positive for methylamphetamines and MDMA respectively, which around 17% are from truck drivers.

On the other hand, to avoid the proposed laws in drug control, manufacturers seek structural changes from chemical synthesis, leading to active compounds with the same effects as existing stimulants, but without knowing the toxicity of new compounds [16]. With the increasing number of new psychoactive substances, it is necessary to constantly search for new detection methods [17,18]. About these, there are methods that seek target compounds such as chromatographic, as well as non-target such as high-resolution mass spectrometry (HRMS) and Nuclear Magnetic Resonance (NMR). The latter two have been widely used in identification of licit and illicit stimulants, such as the work published by Kavanagh [19] which used the HRMS to verify metabolites of two cathinone derivatives in urine, which are widely chemically modified substances. On the other hand, Serrano *et al*. [20] who used NMR even in quantitative mode to determine the 12 compounds in 12 samples seized by the Brazilian Federal Police. In another example, Hussain *et al*. [21] evaluated 20 MDMA samples by NMR in quantitative mode, and the samples were made available by Greater Manchester Police.

Thus, this study aims to identify known and not-known active compounds in ``Rebites``samples used by professional drivers in Brazil. For this, we used HRMS to take a general look at the content of these drugs and later was used NMR to quantify the main active found the caffeine, which were confirmed by HPLC-UV-Vis. In addition, we have analyzed de drugs by Scanning Electron Microscopy (SEM) with elemental analysis by Energy Dispersive Spectroscopy (EDS), to verify the application of this direct analysis in determination of this drugs in tablets.

MATERIALS AND METHODS

All solvents (Acetonitrile, 2-Propanol, Methanol, Dichlorometane) were purchased by Supelco®. In the same hand, the reagents such as: acetonitrile HPLC grade, 2-propanol HPLC grade, methanol HPLC grade, dichloromethane HPLC grade, as well as the caffeine standard were purchased by Sigma-aldrich (Missouri, EUA). Ultra-pure water was obtained from Milli-Q Advantage A10, Millipore®.

Furthermore, 7 real samples of Rebites were collected from various points of Brazil, according to Table 1, between June 2019 and December 2021. The samples were obtained through anonymous donations to contribute with this work. The EST code tablet was used only for mass spectrometry analysis because it did not contain enough sample for the other tests.

Table 1.

SAMPLE PREPARATION FOR HRMS

The tablets were weighed individually, being ground to be diluted in different solvents with distinct polarity degree. Initially, five solvents were tested for extraction: Acetonitrile, 2-propanol, methanol, dichloromethane, and ultra-pure water. For this, about 10 mg of ground tablet were added in five different Eppendorf tubes®, and then 2 mL of solvents was individually added to each Eppendorf®. The tubes were stirred with the aid of a vortex for about 5 min. Subsequently, the suspended phase was filtered, and the resulting solution infused directly into the ionization source.

HIGH-RESOLUTION MASS SPECTROMETER

The tablets were analyzed in a HRMS with an electrospray ionization source (ESI), also composed of a system of mass separation type quadrupole-flight time Q-TOFII (Bruker, Billerica, USA). Prior to infusion of samples, the system was calibrated with a previously prepared solution of 0.01 M sodium formate in water:PrOH (1:1). Then, the samples were diluted in a mixture of acetonitrile:water (1:1) with the addition of 0.1% formic acid and 0.1% of amonium formate for positive and negative mode analyses ESI(+) and ESI(-), respectively. Finally, the samples diluted were injected directly into the ESI source, with the aid of a syringe pump (Bruker®) at a speed of 180 μL/h. Thus, the analyses were conducted in both ESI(+) and ESI(-), with capillary voltage of approximately +4500 V and +40 V for the MS and MS/MS assays respectively. The dessolvatation temperature used was 180°C. For MS/MS mode the ions were tested at different *collision* energies (CID). The analysis range was from *m/z* 50 to 1200 a.m.u, with a velocity of three readings per second for positive mode, generating a resolution of approximately 25,000 for a *m/z* of 200 units of atomic mass. The data were processed using *the Software Data Analysis* (Bruker Daltonics®).

SAMPLE PREPARATION FOR NMR

A caffeine standard purchased from Sigma-Aldrich (Missouri, EUA) with 99.5% was used to confirm the NMR signals. About the samples, it`s were previously ground and about 25 mg of each tablet was weighed directly into the 5mm NMR tube. As internal standard (IS), we

used the reference material produced by Sigma-Aldrich (Missouri, EUA) TraceCERT, exclusive for application in NMR methods: Duroquinone (CAS 527-17-3); bcbr5528V lot valid until 31/04/2023; molecular formula $C_{10}H_{12}O_2$; MM 164.2040; with certified purity of 99.71 ±0.17% (m/m). After, a previously weighed amount with accuracy of Internal Standard (between 2 and 5 mg) was added directly to the NMR tube. This compound was completely solubilized in $CDCl_3$ and for the selectivity presented, being the singlet at 1.94 ppm used for quantification, related to the four methyl groups of this IS. Finally, 500 µL of $CDCl_3$ (sigma-aldrich, USA) was added to the mixture and it was sonicated in ultrasonic bath for 5 min.

RESSONANCE MAGNETIC NUCLEAR (qNMR)

The qNMR method based on Holzgrabe *et al*. [22] was used with modifications, briefly: To determine the content of caffeine in the sample were carried out with experiments using a Bruker NMR spectrometer model *Fourrier 300*, operating at a frequency of 300.13 MHz for ^1H. As solvent was used chloroform deuterated ($CDCl_3$). The spectra were acquired with 64K points and ~11.3 ppm spectral window, which were processed in the TOPSPIN (Bruker®) program, where an exponential multiplication of FIDs was applied by a factor of 0.3 Hz. Chemical shifts for ^1H were calibrated in relation to the residual $CHCl_3$ signal (δ 7.26 ppm). A method was developed for quantitative analyses, using the zg30 pulse sequence, with relaxation time of 1s and mixing time of 100 ms. For each experiment, 32 scans were defined in a total time of 3.5 min, which was suitable for all *spins* were evaluated.

For the calculation of purity, the following formula was used:

$$P_a = \left(\frac{I_a}{I_{Is}}\right) x \left(\frac{N_{Is}}{N_a}\right) x \left(\frac{M_a}{M_{Is}}\right) x \left(\frac{m_{Is}}{m_a}\right) x P_{Is}$$

Where: P_A= Analyte purity; P_{IS} = Internal standard (IS) purity; I_A= Integral area from analyte; I_{IS}= Integral area from IS; N_A= representative nucleus of Analyte; N_{IS}= representative nucleus of IS; M_A = Molar mass of analyte; M_{IS} = Molar mass of IS; m_{Is} = the weight of IS; m_A= the weight of analyte.

PREPARATION OF SAMPLES AND ANALYSIS FOR HPLC-UV-VIS

To caffeine confirmation was used the method determineted by Fajara and Susanti [23], with modifications. The high-efficiency liquid chromatography (HPLC) model was Shimadzu LC-20AD. The system was equipped with a CMB 20A controller, an LC-20AD isocratic pump,

a CTO-20A column oven and an SPD-20A UV/Vis detector Eclipse 5. XDB-C18 column (Zorbax ® HPLC, USA) (250 mm x 4.6 mm, 9 µm particle size) was used to separate the products. The mobile phase used was methanol:milli-Q water (95:5), flow rate of 1.0 mL/min, at 35°C, for 20 minutes, detector set at a wavelength of 278 nm and injection volume of 10 µL. The samples were quantified from the analytical curve with external solution in a linear concentration of 1.9; 3.9; 7.8; 15.0; 31.0; 63.0 and 100.0 µg/ml.

SCANNING ELECTRON MICROSCOPY (SEM) WITH ENERGY DISPERSIVE SPECTROSCOPY (EDS)

Topography of the samples was evaluated by field emission scanning electron microscopy equipment (FEG-SEM, Tescan MIRA3 LMU, Czech Republic) used in high vacuum mode with a maximum beam voltage of 10 kV. Also, the chemical composition was simultaneously verified with coupled silicon drift detector (Oxford Instruments X-act) energy dispersive X-ray spectroscopy (EDS) by means of concentration maps and single-point elemental spectra. EDS spectra, obtained with an energy of 20 keV, were corrected for a thorough investigation of nitrogen presence and simulation of elemental concentration in nitrided samples through NIST DTSA-II software following methodologies guided in accordance with Ritchie.

Maximum EDS analysis depths were estimated through Monte Carlo simulations, carried out with the software CASINO v3.3: 700 nm. The sample was fixed in the sample holder with the aid of double-sided tape based on conductive carbon (EMS - Pennsylvania - USA). Then, the surface was covered with a conductive gold film. The deposition method was the Magnetron Sputtering (Plasma Deposition), with a current of 30mA and for 30s. For the analysis, the following conditions were used: 10kv acceleration voltage; the working distance was 10 mm and the detector was (BSE) Backscattered Electrons, in order to avoid static loading of the particles as they are very irregular in shape and size. For qualitative energy scattering X-ray microanalysis (EDS), a Silicon Drift Detector type detector was used (SSD - XMax-50 Oxford Instruments). The conditions were as follows: 20 kV acceleration voltage; beam intensity of 16 and the working distance was 15 mm.

RESULTS AND DISCUSSION

The HRMS is a versatile analytical technique for the characterization of organic of target and not-target compounds, which has been highlighted for identification of non-target compounds relevant to the forensic area [24]. Among the advantages this the minimum or no need to sample preparation, which after dilution could be directly infused into an ESI ionization source to have an overview of chemical composition. In this sense, as we expected to find compounds with different physicochemical characteristics, the tablets were diluted with polarity different solvents compatible with HRMS-ESI. For this, we used the VGL as a prototype, which was weighed and dissolved, and the resulting solution was injected. The diagnosed ions were analyzed according to their exact *m/z* with maximum 10 ppm error [25]. Other characteristics, such as isotopic ratio and fragmentation profile are also used to confirm the elemental composition as well as the molecular structure.

As result, in ESI(+) we observed the presence of the same cations in all spectra, with the ratio *m/z* 260 being the base peak, here represented by spectrum carried-out from sample diluted in acetonitrile Figure 1A (Figure 1S, support information shown all). Meanwhile, in ESI(-) mode, there is a difference in spectra's, mainly related to the intensity of anions (Figure 2S, support information shown all). However, for this work we identified the anions obtained mainly from the extraction with acetonitrile by the difference of accuracy and sensitivity for the other, Figure 1B. In this line, we have used this solvent for analysis of the other samples.

Figure 1.

ION'S DIAGNOSTIC - ESI(+)

The most abundant cations present in the VGL tablets were with *m/z* 195 and 260 which is proposed to be related to molecular formulas $[C_8H_{10}N_4O_2 + H]^+$ and $[C_{16}H_{18}ClN + H]^+$, respectively, which were determinate as caffeine (Figure 2) and clobenzorex (Figure 3) respectively. While the first is a compound known as CNS stimulant, being among the most commercialized in clandestine formulations [26], the second is also a stimulant as well as an appetite suppressant banned in Brazil [27]. The ions with *m/z* 91, 119 and 142 correspond to the fragmentation process at the source of clobenzorex, which have generated the cations $[C_7H_7 + H]^+$, $[C_9H_{11} + H]^+$ and $[C_7H_6Cl + H]^+$, respectively. In the same side, the ion with *m/z* 557 is a clobenzorex dimer $[C_{16}H_{18}ClN + C_{16}H_{18}ClN + H]^+$. However, the cations with *m/z* 109, 112,

165 and 179 are related a to source fragmentation of caffeine, as well as the with *m/z* 217 is a sodium adduto $[C_8H_{10}N_4O_2 + Na]^+$.

In a recent study conducted by Cunha *et al*. [28], 50 blood samples obtained from the Institute of Legal Medicine of Sergipe were evaluated and the authors detected, through analysis by LC-MS/MS, the presence of clobenzorex in a blood sample related to a truck traffic accident. Already the authors Souza *et al*. [26] evaluated ecstasy pills seized by the Police in the state of Santa Catarina from 2011 to 2017 and found these substances, caffeine and clobenzorex, in the pills. The most expressive number of pills containing these compounds together was in 2013, with more than 1500 pills.

Figure 2.

Figure 3.

The ESI(+) mass spectra of DSB, CJE, EST and AFT have shown similar profile of the VGL (Figure 3S until 8S). In addition, EST presented ion 235 which was related to lidocaine ($[C_{14}H_{22}N_2O + H]^+$), used as anesthetic and antiarrhythmic [12]. In the same sample the *m/z* 475, was related to sildenafil citrate $[C_{22}H_{30}N_6O_4S + H]^+$, popularly known as Viagra®, which is used for treatment in erectile dysfunction and pulmonary hypertension [12]. The use of drugs for erectile dysfunction aims to reduce the adverse vasoconstrictor effects caused by amphetamines on sexual performance [29]. In other side, the AMR sample presented *m/z* 256 $[C_{17}H_{21}NO + H]^+$ and the fragment with *m/z* 167 $[C_4H_{10}NO + H]^+$, which were related with phenyltoloxamine an antihistaminic belonging to the ethanolamine group, which has a euphoric effect and can cause pain reduction [30].

Antihistamines are compounds that appear in a study related to drug abuse and traffic accidents, or even airplanes, as is the case of study reported by McKay and Groff [31], which had evaluated the toxicological tests of U.S. aircraft pilots involved in air accidents between the years 1990 to 2012. This study found the use of another antihistamine, the diphenhydramine and classified several medications as to their ability to be potentially harmful. Already a work carried out by Tomaszewski *et al*. [32] evaluated 242 urine samples from drivers, where in two samples the use of antihistamines was also identified, and phenyltoloxamine is among the possible substances.

ION'S DIAGNOSTIC - ESI(-)

Meanwhile, in negative mode, the main anion presents in all samples tested, have shown the *m/z* 213, which is relative 8-chlorothephylin $[C_7H_6ClN_4O_2 + H]^+$, Figure 4. Theophylline and its analogues belong to the group of CNS stimulants and are present in various beverages such as teas and coffees. According to Rang [12], "performance in motor tasks, such as typing and driving in a simulated way, also improves, particularly in tired individuals". The reduction of tiredness, the improvement in concentration can be a justification for the use of these compounds widely distributed among professional drivers in Brazil [12]. Ions 128, 179 and 181 are fragments of 8-chlorothephiline, resulting from losses of $[C_3H_3NO_2 - H]^-$, $[Cl - H]^-$ and $[OCH_3 - H]^-$, respectively.

Figure 4.

Theophylline has been reported in several studies for several years, such as in the study by Kinberger *et al*. [33] already aimed to evaluate theophylline in urine and blood to perform toxicological tests on drivers involved in traffic accidents. From this the authors developed a method capable of detecting the compound by gas and liquid chromatography. McKay and Groff [31] also had indicated theophylline as Potentially impairing. Thus, 6 different compounds were identified in both modes, which are indicated in Table 2.

Table 2.

USE OF QNMR FOR CAFFEINE QUANTIFICATION

Among the advantages of the use of NMR to characterize mixtures is the possibility of identification of non-target compounds. In this sense, in 2014 Johansson *et al*. proposed a platform based on analytical tools for the identification of illegal compounds, among which the NMR was highlighted by its characteristics. When we analyzed Rebite samples, we confirmed that the main component is caffeine and clobenzorex, Figure 5 for VGL.

Figure 5.

Another important feature of NMR is the application to quantify compounds in complex mixtures. In this sense, we have recently reported studies that make use of qNMR for quantification of illegal formulations of glyphosate, other pesticides such as azoxystrobin, as well as for quality control of coumarin-based in pharmaceuticals and plant medicines. Here, we have used a caffeine standard to confirm the chemical shift of signals (Figure 3S. support information), as well as the signals of duroquinone (IS) was confirmed before use (Figure 4S. support information), which the signals in N-CH_3 in 3.98 ppm and the four methyl groups in 1.99 ppm were used for quantification respectively, Figure 6.

Figure 6.

Experimentally, five measurements were performed for each sample, being the sample and the IS individually weighed and subsequently diluted directly in the NMR tube with 500 μL of $CDCl_3$. The results are presented in Table 3, where the amount of caffeine for the VGL sample was determined in % (m/m). Meanwhile, table 4 shows the result obtained for the other 5 samples.

Table 3.

Table 4.

The NMR technique is a technique that has been used in drug quality control, as for instance Santos and Colnago [34] had used the qNMR to evaluate and quantify the presence of paracetamol in commercial tablets. Furthermore, nuclear magnetic resonance imaging is being disseminated in the forensic sphere since it can identify new psychoactive substances. As demonstrated by the study conducted by Leite [35], which reported the cannabinoids present in marijuana samples s seized in the state of Espírito Santo. In addition, the authors have also used the gas chromatography, to confirm the structures and compounds found by NMR.

USE OF HPLC-UV FOR CAFFEINE QUANTIFICATION

Here, the caffeine was also analysed by HPLC-UV. The standard curve was determinate with a coefficient (R^2) of 0.9994 ($y = 25206x + 22877$). The table 5 had demonstrated the content of caffeine present in these six samples.

Table 5.

In other side, Aheampong *et al*. [36] have quantified simultaneously caffeine, chloropheniramine maleate and paracetamol in tablets by HPLC-UV with reverse phase C18 column. Using internal standard method, the compounds were effectively determinate in concentrations ranging 0.1 to 12.5 $mg.mL^{-1}$ in commercial tablets.

CHEMICAL COMPOSITION BY SEM-EDS

The SEM-EDS has been applied to forensic chemistry such as for elementary composition determination of Shooting events in Kosovo by Tahirukaj *et al*. [37]. In other hand, polymorphism and elemental composition have been related using the same technique as the reported by Pajula *et al*. [38] for tablets containing Indomethacin, Terfenadine, Paracetamol, Naproxen and Cimetidine. In order to identify a possible application of the technique for these tablets, the chemical composition by EDS was carried out simultaneously with the SEM topography imaging from the sample DSB. In Figure 7, we can see the microscopy evaluation ranging from 2,000 to 10,000 with the solids size measurements (Figure 7 A-B). Furthermore, the elemental composition was determined in 10 distinct points (Figure 7 - C), and three representative spectra were presented (Figure 7 D-E).

The semi-quantification of the elemental composition is indicated in Table 6, where we have an average of the % mass of Carbon, Nitrogen, Oxygen and Chlorine in the specific positions. From the results we observed that spectrum 13 presents an elemental amount of chlorine higher than the others and may be related to chlorobenzorex ($C_{16}H_{18}ClN$) which has elemental composition: C - 73.98; H - 6.98%; Cl - 13.65%; N - 5.39%. In other side, the other measures we have a theoretical elemental composition like caffeine ($C_8H_{10}N_4O_2$): C – 49.48%; H - 5.19%; N - 28.85%; O - 16.48%. It is worth noting that from this technique atoms like hydrogens are not determined.

Table 6.

Here we have demonstrated the applicability of the technique for determination of small organic compounds, such as the one presented here for tablets containing a mixture of drugs. In the same line, this method has already been used to determine purity in some drugs, as demonstrated by Luiz *et al*. [39], which compare potassium diclofenac, generic and similar tablets. Furthermore, the technique has been disseminated in the forensic area for analysis of

gunshot residues. Weber *et al*. [40] were able to identify the chemical compounds present in the gunpowder.

In Brazil, the economic pressure causes professional truck driver working by exhausting working hours, which reflects in increasing use of stimulant substances. As reported by Oliveira *et al*. [41] between 180 truck drivers tested in São Paulo state, were found the prevalence of the psychostimulant as amphetamines and cocaine or their metabolites. Around the world different drugs has been used for drivers as: Lema-Atán *et al*. [42] in Spain, which has detected cannabis as the most consumed drug, cocaine, amphetamines, heroin and ketamine; Barone *et al*. [43] indicated cannabis as the most common illicit drug found in toxicological tests of injured drivers as well as cocaine and amphetamines in drivers from Belgium, Denmark, Finland and Norway. In Germany, amphetamines were present in 12.6% of the positive tests performed on 837 drivers, being the second most consumed drug between 2017 and 2018. Other illicit drugs such as THC, benzoylecgonine (cocaine metabolite), MDMA, methamphetamine and morphine were also detected in toxicological tests [44].

In summary, in this work we demonstrate the analysis of tablets which are easily accessed by professional drivers in gas station, used regularly used to keep them on alert. In the first stage we qualified the chemical composition by high resolution mass spectrometry, which assisted in the identification of two compounds in ESI(-) mode and six compounds in ESI(+). From this, we identified analgesics as lidocaine and in addition to stimulants such as theophylline and chlorobenzorex. In all samples analyzed, the main active compound found was caffeine, which was quantified by NMR and HPLC-UV. To contribute to the chemical characterization, the tablets were also analyzed by SEM-EDS. In comparison by NMR the caffeine content found was between 14.57 and 30.57, by HPLC-UV between 11.18 and 29.06. These results confirm that the qNMR is effective and reliable, despite the need for some adjustments in the analytical method, indicating that the technique can be used in the forensic scope for determination of grades. Furthermore, this difference in results may be associated both with the preparation of the sample for the HPLC technique and with the equipment itself, in addition to the difference in sensitivity of the two techniques and the interference of excipients.

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TABLE CAPTIONS

Table 1. Rebites samples collected around Brazil.

Sample	Acronym	City
Vagalume	VGL	Anápolis – Goiás
Desobési	DSB	Araguaína – Mato Grosso do Sul
Corujão Extra Forte	CJE	Ibitira – Bahia
Coruja Original	CJO	Ibitira – Bahia
Amarelo	AMR	Brejões - Bahia
Águia Forte	AFT	Brejões - Bahia
Estrela	EST	Cachoeira dos Índios – Paraíba

Table 2. Compounds identified in samples analyzed by HRMS

Compound	Sample	Molecular formula	m/z	Error (ppm)	Isotopic ratio (mSig)	Fragmentation Profile
Isoniazid	AFT	$C_6H_7N_3O$	138.0662	1.7	2.7	-
Caffeine	VGL, CJO, CJE, DSB, AFT	$C_8H_{10}N_4O_2$	194.0876	0.2	5.2	*m/z* 109.0640 [M - $C_3H_4NO_2$]$^+$ *m/z* 112.0631 [M - $C_3H_3N_2O$]$^+$ *m/z* 165.0538 [M - CH_4N]$^+$ *m/z* 179.0927 [M - CO]$^+$ *m/z* 217 [M - $C_8H_{10}N_4O_2$ + Na]$^+$
8-chlorothephiline	VGL, CJO, CJE, DSB, AMR,	$C_7H_6ClN_4O_2$	214.0258	9.70	4.3	*m/z* 128 [M - $C_3H_3NO_2$]$^-$ *m/z* 178 [M - Cl]$^-$ *m/z* 181 [M - OCH_3]$^-$
Lidocaine	VGL, CJE, DSB, AFT, EST	$C_{14}H_{22}N_2O$	234.1732	2.20	100.7	-
Phenyltoloxamine	CJE, AMR	$C_{17}H_{21}NO$	255.1623	11.10	256.3	*m/z* 167 [M - C4H10NO]+
Clobenzorex	VGL, CJO, CJE, DSB, AFT	$C_{16}H_{18}ClN$	259.1128	4.10	16.4	*m/z* 91.0548 [M - C_7H_7]$^+$ *m/z* 119.0861 [M - C_9H_{11}]$^+$ *m/z* 142.0417 [M - C_7H_6Cl]$^+$ *m/z* 557 [M - $C_{16}H_{18}ClN$ + $C_{16}H_{18}ClN$]$^+$
Sildenafil	VGL, CJO, EST	$C_{22}H_{30}N_6O_4S$	474.2049	1.10	26.3	-

Table 3. Calculations of the caffeine content via qNMR for the VGL sample using IS.

Entry	IS (mg)	Sig. area.	nH. IS	Sample (mg)	Sig. area.	nH Sample	Caffeine	Media
1	2.11	12170931213	12	21.31	3733704855	3	14.33	14.57
2	2.35	14598685938	12	21.85	4252570916	3	14.78	**SD**
3	2.55	15505628196	12	23.01	4457000186	3	15.03	0.32
4	2.93	16512041438	12	22.97	3927214518	3	14.31	
5	2.75	15534532216	12	23.73	4089737060	3	14.39	

Table 4. Results of the caffeine content via qNMR for the others samples using IS

Sample	Caffeine concentration (%)	SD
VGL	14.57	0.32
CJO	30.57	0.36
CJE	19.24	0.38
DSB	27.60	0.37
AFT	25.25	0.21

Table 5. Results of the caffeine content via HPLC.

Sample	Concentration (%)
VGL	14.85
CJO	29.06
CJE	12.46
DSB	11.18
AMR	27.75
AFT	15.51

Table 6. Chemical composition determinate by SEM-EDS.

Entry	C (%)	N (%)	O (%)	Cl (%)	Total (%)
Spectrum 11	45.55	32.44	21.81	0.19	100
Spectrum 12	48.17	30.79	20.76	0.28	100
Spectrum 13*	67.69	13.67	11.86	6.78	100
Spectrum 14	49.43	29.36	21.21	-	100
Spectrum 15	45.03	34.11	20.86	-	100
Spectrum 16	46.01	31.25	22.74	-	100
Spectrum 17	47.43	30.48	22.09	-	100
Spectrum 18	52.11	27.18	20.72	-	100
Spectrum 19	44.51	34.17	21.32	-	100
Spectrum 20	46.13	33.43	20.44	-	100
Average*	47.15	31.47	21.33	0.24	100
Stdv*	2.43	2.33	0.75	0.06	100

*Except the spectrum 13.

FIGURES CAPTIONS

Figure 1. Mass spectra in ESI(+) mode in (**A**) and ESI(-) in (**B**) of the VGL sample diluted in acetonitrile.

Figure 2. Comparison of exact *m/z* and isotopic ratio for ion with *m/z* 195.0876, determined as caffeine

Figure 3. Comparison of exact *m/z* and isotopic ratio for ion with *m/z* 260.1227, determined as clobenzorex

Figure 4. Comparison of exact *m/z* and isotopic ratio for ion with *m/z* 213.0195, determined as clobenzorex

Figure 5. NMR spectrum for sample VGL

Figure 6. NMR spectrum for sample AFT

Figure 7. FESEM-EDS analysis for DSB sample. In **A**) the topography of the sample with an increase of 2,000x; in **B**) the topography of the tested sample with an increase of 10,000x with the size measurements; in **C**) the sampled points; and from **D**) to **F**) the elemental analysis for spectrum 11, 12, and 13 respectively

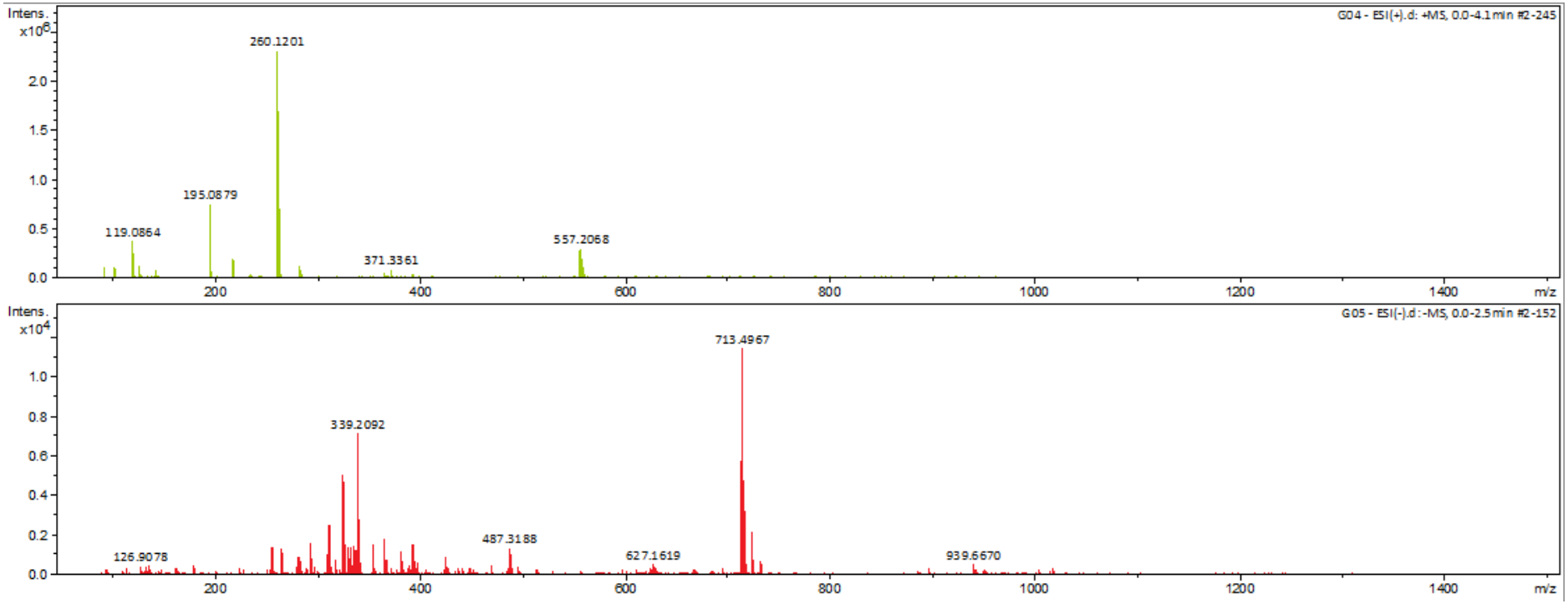


Figure 1.

*Where A) ESI(+) mode and B) ESI(-) mode

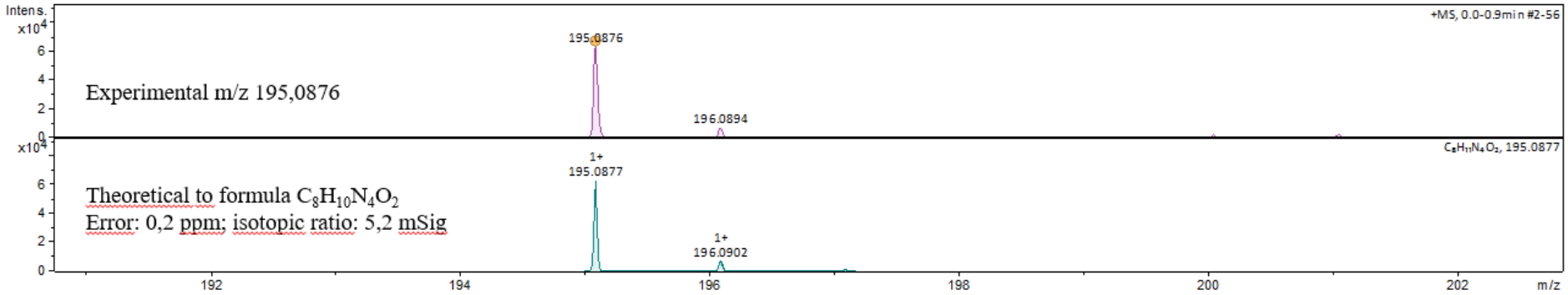


Figure 2.

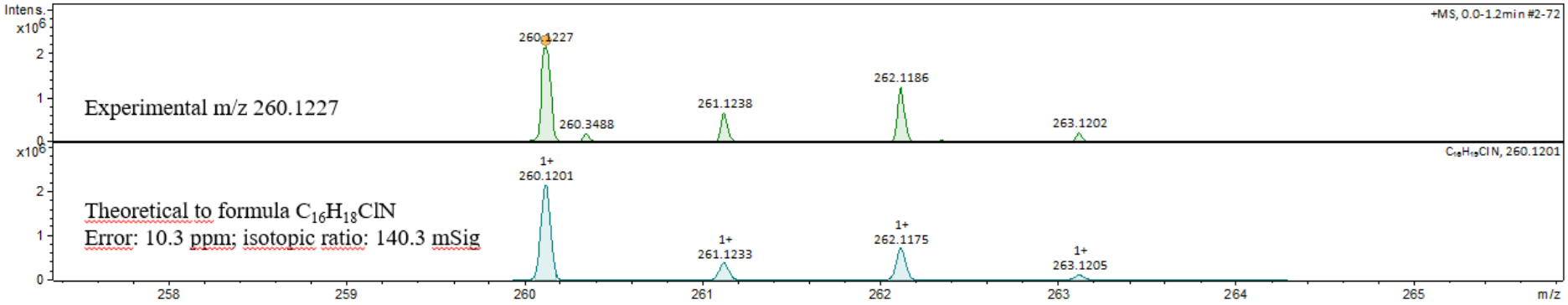


Figure 3.

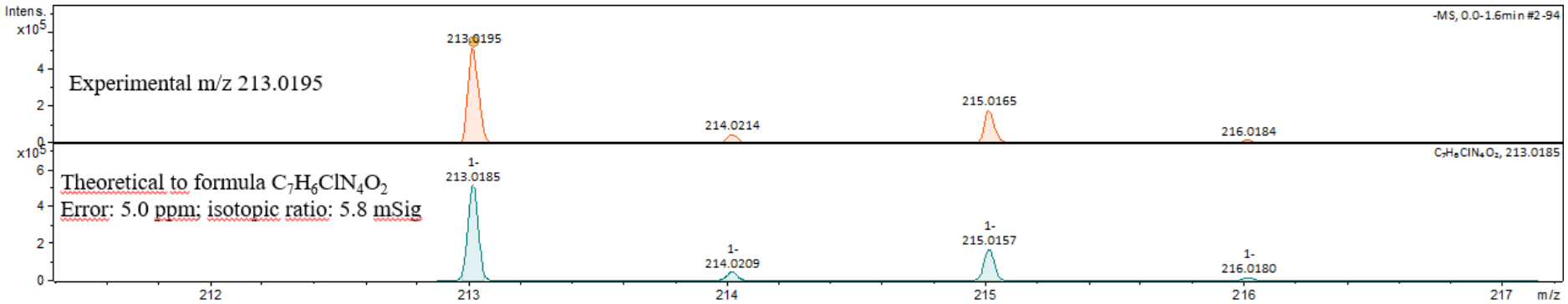
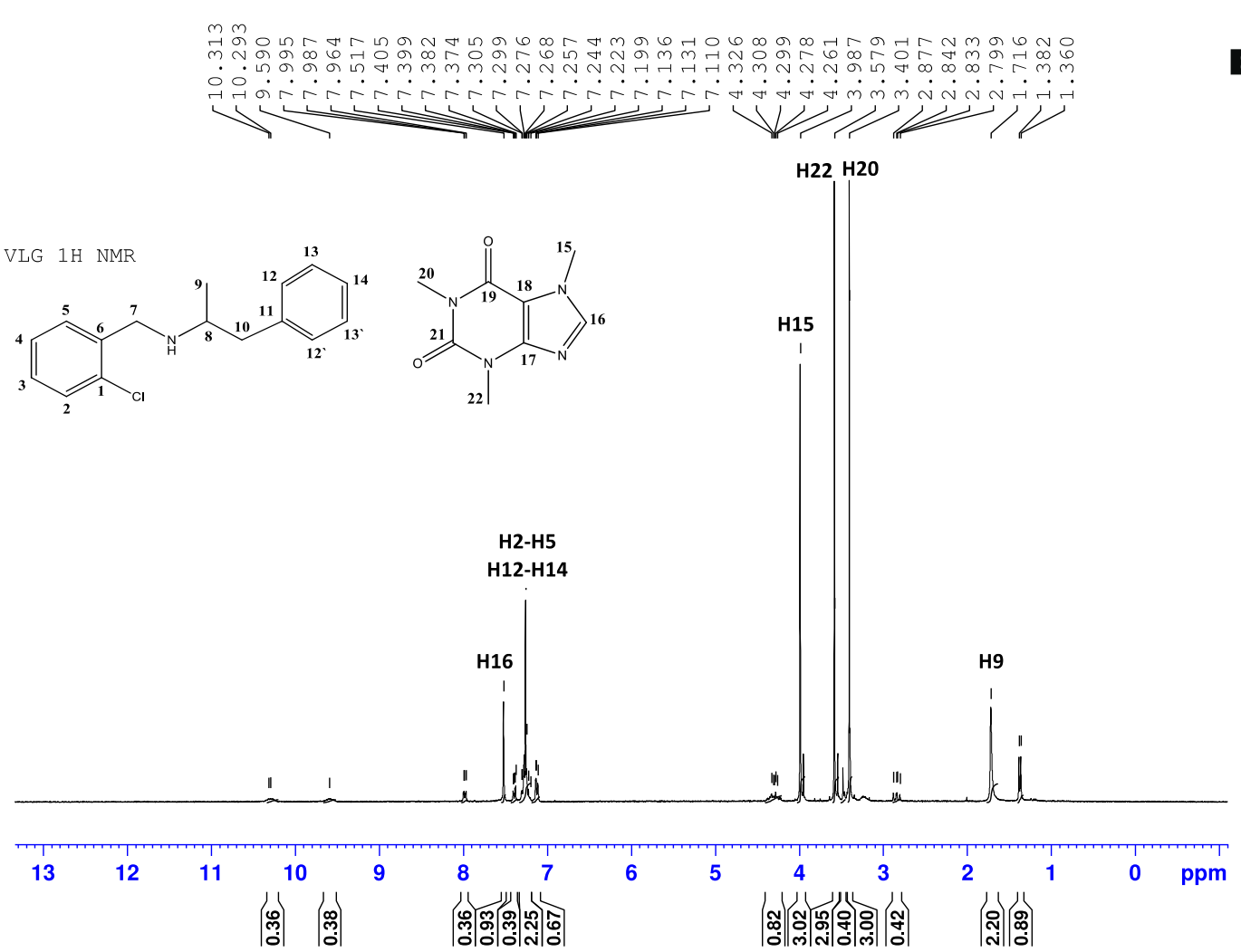

Intens.
x10^5
-MS, 0.0-1.6min #2-94
213.0195
Experimental m/z 213.0195
214.0214
215.0165
216.0184
x10^5
C7H6ClN4O2, 213.0185
Theoretical to formula C7H6ClN4O2
Error: 5.0 ppm; isotopic ratio: 5.8 mSig
1-
213.0185
1-
214.0209
1-
215.0157
1-
216.0180
212
213
214
215
216
217
m/z

Figure 4.

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PROCNO                     1

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PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       13
DS                        0
SWH                6103.516 Hz
FIDRES            0.093132 Hz
AQ                5.3687091 sec
RG                   31.623
DW                   81.920 usec
DE                    6.50 usec
TE                   290.2 K
D1              1.00000000 sec
TD0                       1
SFO1           300.1818537 MHz
NUC1                     1H
P1                   29.75 usec
PLW1           11.00000000 W

F2 - Processing parameters
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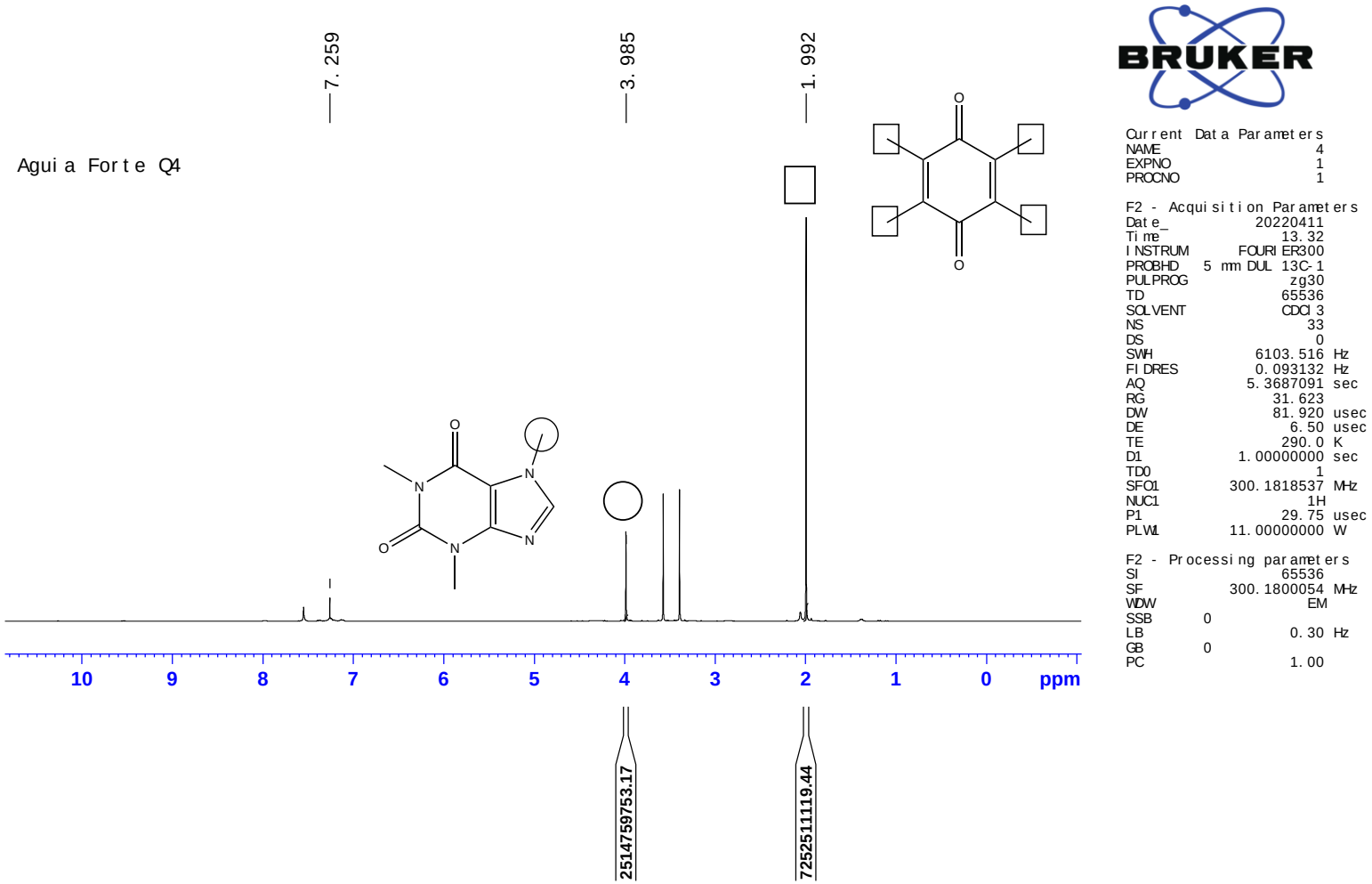
WDW                      EM
SSB         0
LB                    0.30 Hz
GB          0
PC                    1.00
```

Figure 5.



BRUKER

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PROCNO 1

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PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 33
DS 0
SWH 6103.516 Hz
FIDRES 0.093132 Hz
AQ 5.3687091 sec
RG 31.623
DW 81.920 usec
DE 6.50 usec
TE 290.0 K
D1 1.00000000 sec
TD0 1
SFO1 300.1818537 MHz
NUC1 1H
P1 29.75 usec
PLW1 11.00000000 W

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WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Figure 6.

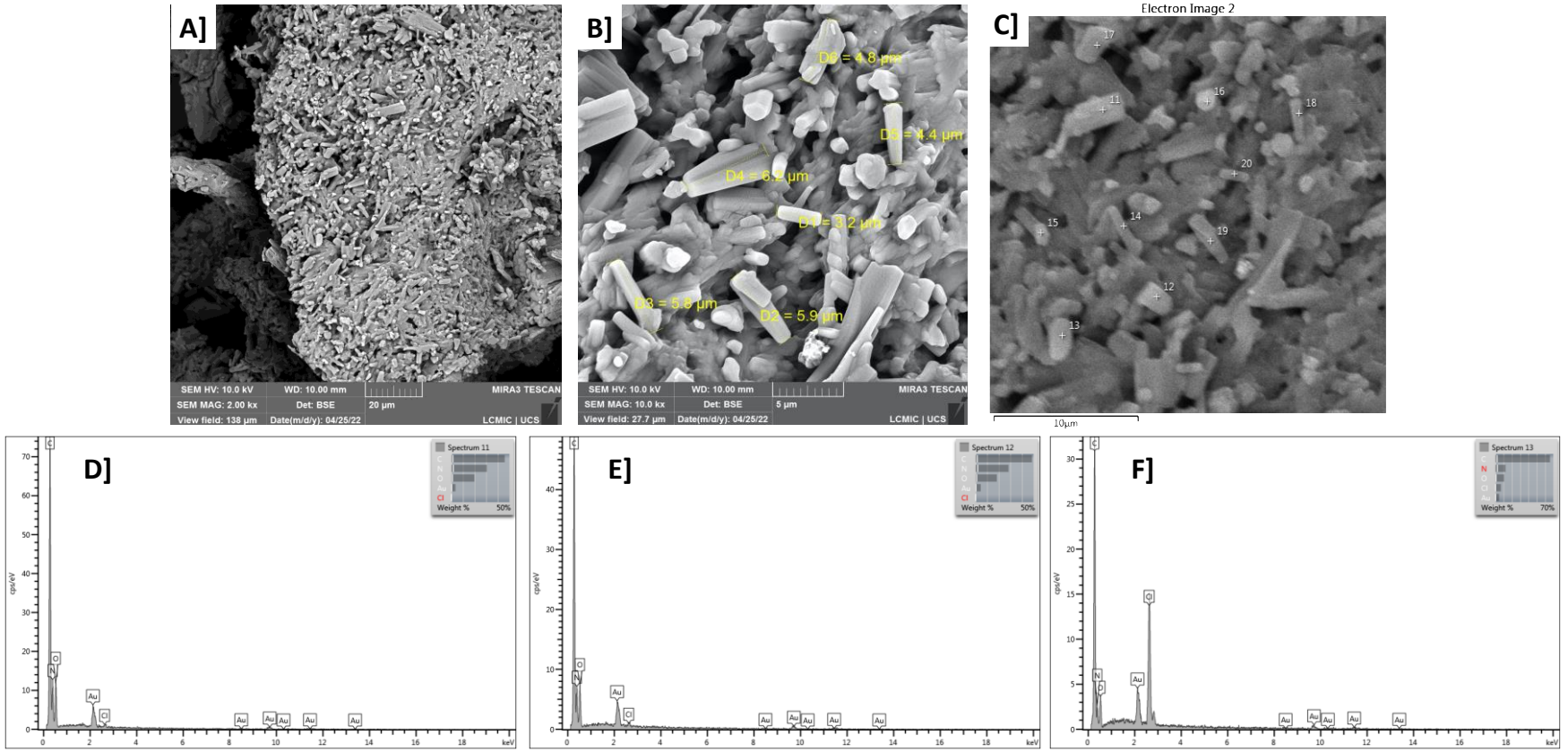
A]
B]
C]
D]
E]
F]
D6 = 4.8 µm
D5 = 4.4 µm
D4 = 6.2 µm
D1 = 3.2 µm
D3 = 5.8 µm
D2 = 5.9 µm
Electron Image 2
10µm
SEM HV: 10.0 kV
WD: 10.00 mm
MIRA3 TESCAN
SEM MAG: 2.00 kx
Det: BSE
20 µm
View field: 138 µm
Date(m/d/y): 04/25/22
LCMIC | UCS
SEM HV: 10.0 kV
WD: 10.00 mm
MIRA3 TESCAN
SEM MAG: 10.0 kx
Det: BSE
5 µm
View field: 27.7 µm
Date(m/d/y): 04/25/22
LCMIC | UCS
Spectrum 11
Spectrum 12
Spectrum 13
Weight %
cps/eV
keV

Figure 7.

DISCUSSÃO GERAL

O uso de substâncias psicoativas tem grande impacto no número de acidentes no trânsito, principalmente nos que envolvem caminhoneiros, visto que o uso de estimulantes aumenta o risco de acidentes fatais cinco vezes mais quando comparados aqueles com motoristas não usuários (OPAS, 2019a). O aumento do uso de estimulantes por esses profissionais se deve principalmente pelas longas jornadas de trabalho que, devido a pressão econômica tem causado uma nos prazos entregas (TAKITANE, 2013). Entre estes compostos podemos destacar o uso recorrente de estimulantes como anfetamínicos, cocaína e seus derivados.

No âmbito forense surgem diversas drogas novas todos os dias, além de drogas conhecidas serem modificadas estruturalmente, com o intuito de burlar a legislação e a fiscalização dos órgãos responsáveis. Contudo, apesar dessas drogas apresentarem efeitos semelhantes a compostos já conhecidos, o abuso dessas substâncias pode levar a graves intoxicações, as quais podem ser fatais colocando em risco a saúde do usuário (DAÉID, 2016; MAURER; BRANDT, 2018). Uma vez que as identificações realizadas são de importância vital para descobrir essas variantes nas estruturas e com a falta de informações sobre elas como sua toxicologia. É de extrema importância técnicas analíticas capazes de identificar a composição da droga, seja composto alvo ou não-alvo (esperado ou não), seja em drogas apreendidas ou até mesmo em matrizes biológicas do usuário requeridas pela polícia.

A Espectrometria de Massas de Alta Resolução (EMAR) é uma das técnicas mais difundidas no âmbito forense para análise de drogas, isso porque tem uma alta sensibilidade sendo capaz de detectar traços de drogas. Ainda, a técnica possui um curto tempo de análise, um preparo de amostra simples e requer uma quantidade mínima da amostra (ROMÃO, 2010). Além disso, é eficaz para qualificar compostos alvo e não-alvo, seja em amostras da droga bruta ou em matrizes biológicas, sendo elas alternativas ou não.

O Capítulo I demonstra vários trabalhos realizados com espectrometria de massas nas matrizes biológicas alternativas de cabelo e fluído oral, aonde os autores foram capazes de identificar diversas drogas de abuso através da técnica. Ao todo foram 21 trabalhos estudados, dentre eles 7 utilizaram fluído oral como matriz biológica e 14 utilizaram cabelo como matriz biológica. A maioria dos analitos avaliados pelos autores são anfetamínicos, contudo alguns autores como Strano-Rossi et al. (2012) identificaram

NPS, tais como as substâncias conhecidas como “JWH”. Já Montesano, Johansen e Nielsen (2014) avaliaram 96 classes diferentes de drogas. Ainda em todos os trabalhos foram realizadas a quantificação dos analitos através da técnica de cromatografia à líquido, a qual é comumente utilizada no âmbito forense para quantificação de drogas.

Técnicas quantitativas, bem como os equipamentos utilizados, como a cromatografia a líquido de Alta Eficiência, possuem certas limitações, tais como serem destrutivas com a amostra, requerer um preparo de amostra muitas vezes complicado e a amostra entra em contato com o equipamento. Essas limitações podem ser supridas pela Ressonância Magnética Nuclear, ainda que a técnica possua suas próprias limitações tais como sua baixa sensibilidade frente a técnica de espectrometria de massa. Ainda que haja um custo elevado atrelado tanto ao equipamento quanto a sua manutenção, sua utilização em análises de rotina como em laboratórios forense, se torna mais viável que análises por cromatografia a líquido. Isso se deve ao baixo custo na obtenção de um único espectro, levando em consideração gastos atrelados com consumíveis no preparo de amostra e no curto tempo de análise (LEITE, 2013).

A técnica de qRMN se mostrou capaz de substituir a técnica de cromatografia a líquido na quantificação de drogas já conhecidas, além de novas drogas psicoativas. Contudo, ainda se faz necessário o uso conjunto da técnica de espectrometria de massas para avaliar os compostos alvo e não-alvo nas amostras apreendidas. Com a utilização das duas técnicas de forma conjunta, é possível que as análises se tornem cada vez mais rápidas tendo em vista que ambas as técnicas tem um curto tempo de análise. Além disso, o custo para realizá-las seria reduzido visto que ambos os preparos de amostra são mais simples que a cromatografia a líquido.

O Capítulo II demonstra as vantagens de utilizar ambas as técnicas em conjunto, aonde pela técnica de espectrometria de massas foi possível identificar diversos compostos como clobenzorex e ainda verificar que em todos os comprimidos avaliados havia a presença de cafeína. A partir disso, foi possível quantifica-la por RMNq indicando que os comprimidos continham de 14 a 30% de cafeína na sua composição. A fim de verificar a veracidade dos dados obtidos por RMNq foi realizada as análises por HPLC, aonde com alguns ajustes no método analítico será possível indicar tanto a veracidade dos resultados quanto a confiabilidade da técnica de RMNq.

CONCLUSÕES

A partir desse trabalho foi possível identificar que a ressonância magnética nuclear quantitativa é uma ótima alternativa no âmbito forense, principalmente nas análises de rebites onde seu controle por parte de órgãos competentes, bem como leis é dificultado, visto que não há uma regulamentação para os mesmos, nem um controle de qualidade. Além disso, a RMNq tem vantagens frente a outras técnicas que são interessantes para a Polícia Federal, um exemplo disso é a não degradação da amostra, possibilitando o armazenamento da mesma para análises de contraprova, por exemplo. Para verificar a confiabilidade e veracidade dos resultados obtidos por RMNq e contribuir com a caracterização química, os comprimidos também foram analisados por HPLC-UV e SEM-EDS. Ainda, se faz necessário o uso conjunto da técnica de espectrometria de massas a fim de identificar compostos alvo e não-alvo seja em amostras da droga bruta ou em matrizes biológicas.

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DETERMINAÇÃO DE COMPOSTOS NÃO-ALVO POR HRMS E QUANTIFICAÇÃO DE CAFEÍNA POR RMN EM DROGAS USADAS POR MOTORISTAS PROFISSIONAIS NO BRASIL

TAINARA GUIZOLFI

APÊNDICE A – ESPECTROS DE MASSAS DAS AMOSTRAS DE REBITES

ÍNDICE

Figura 1 - Espectros de EMAR para a amostra VGL

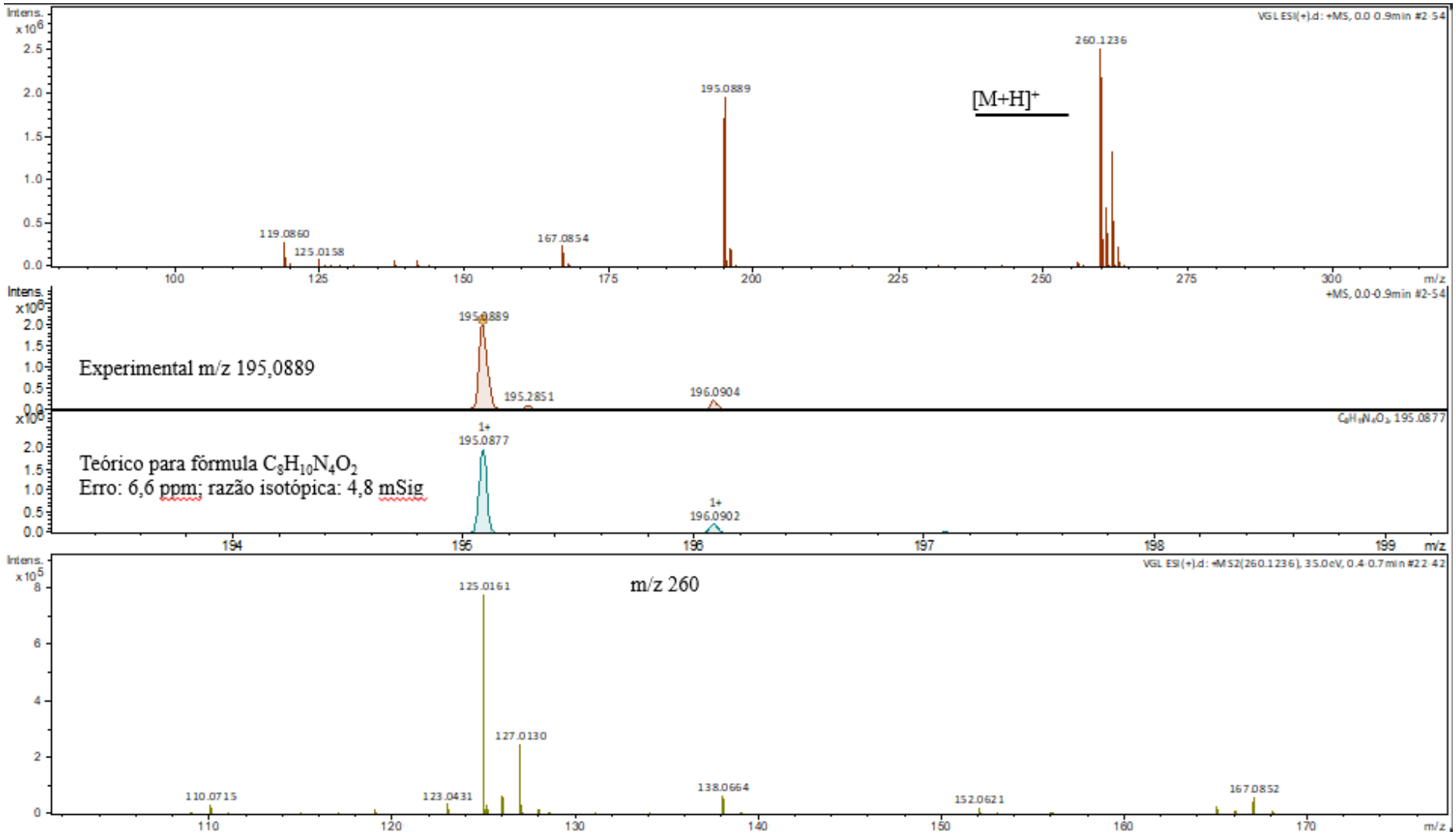


Figura 2 - Espectros de EMAR para a amostra DSB

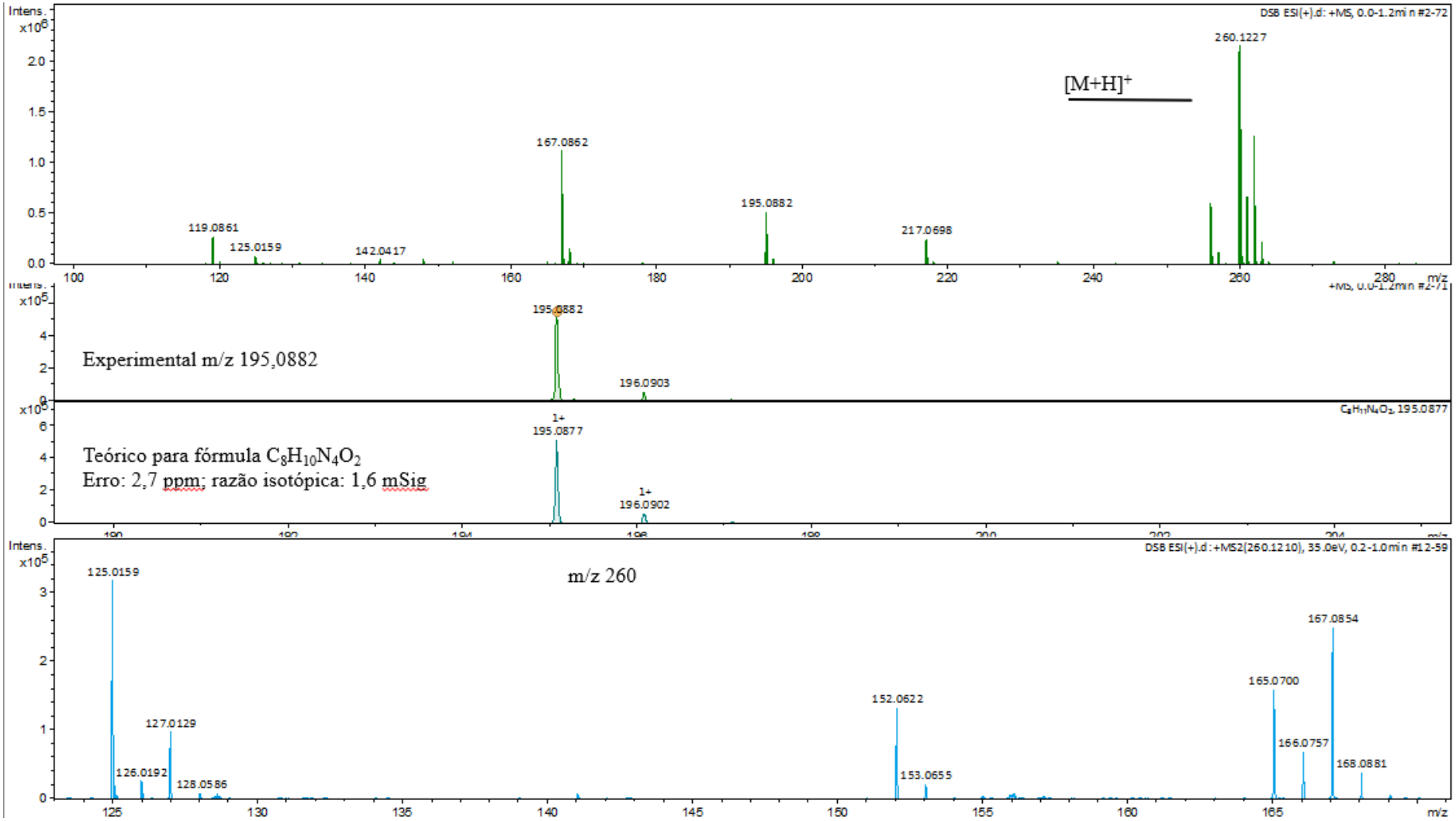


Figura 3 - Espectros de EMAR para a amostra CJE

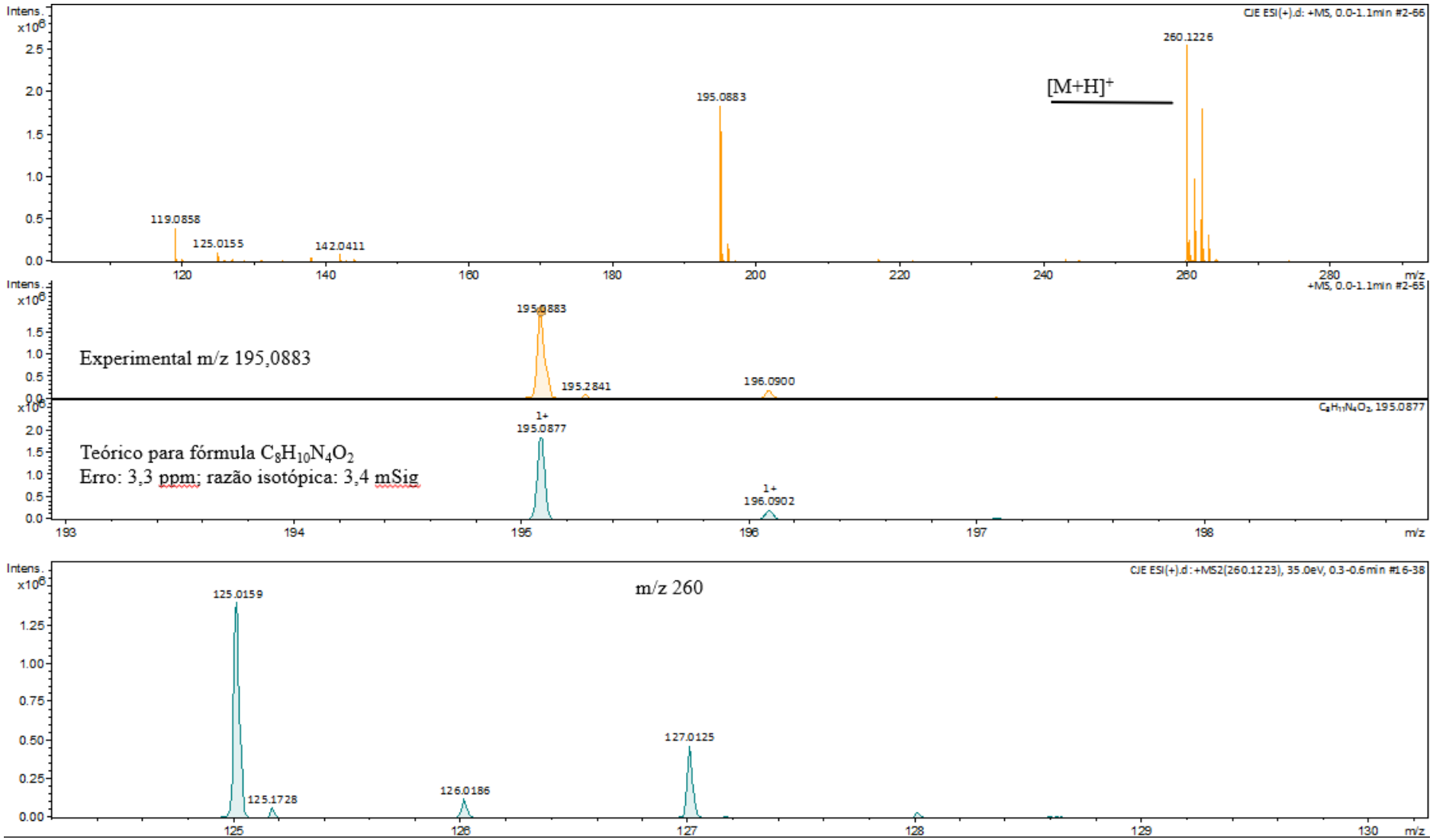


Figura 3 - Espectros de EMAR para a amostra CJO

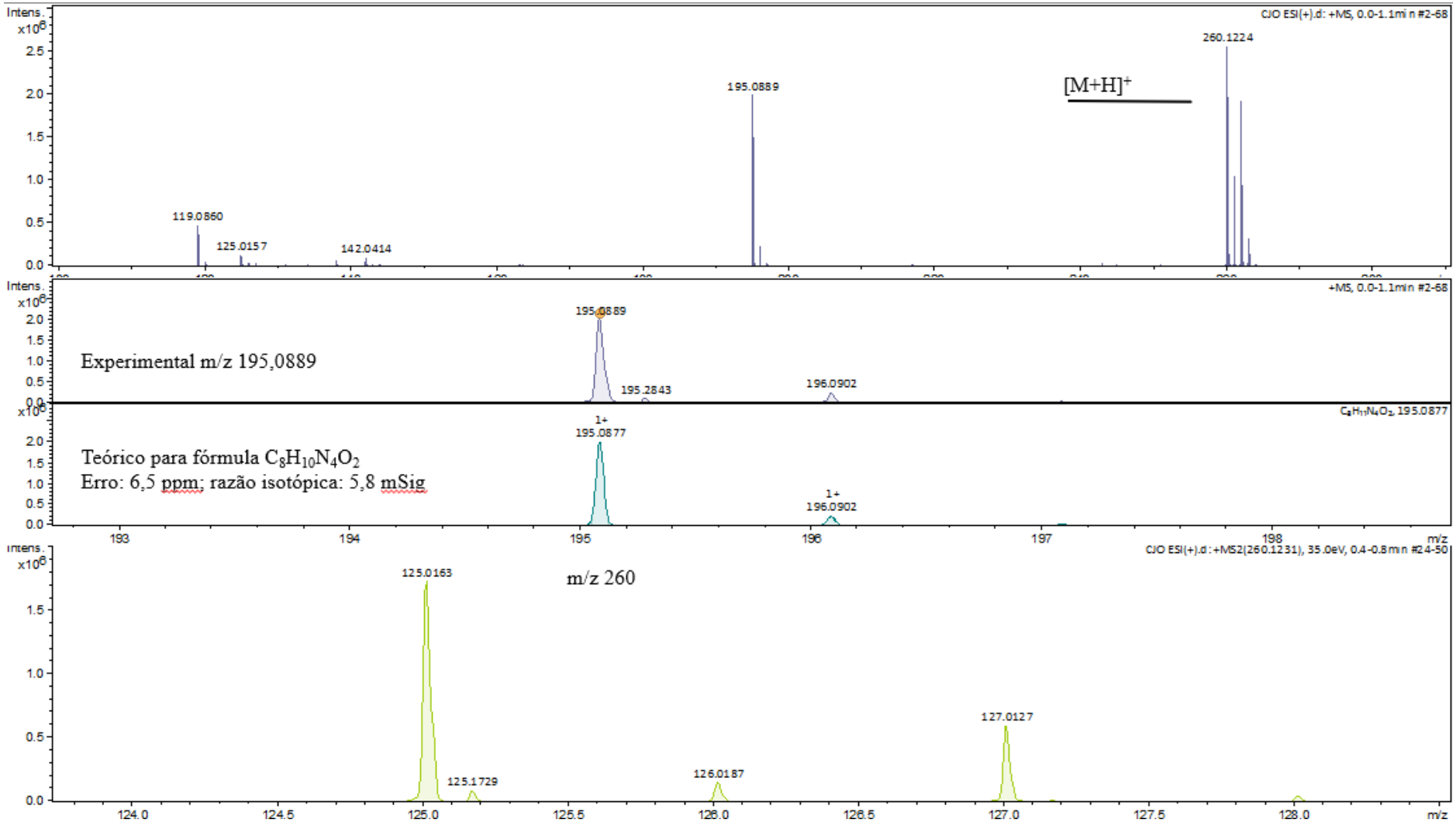


Figura 4 - Espectros de EMAR para a amostra AMR

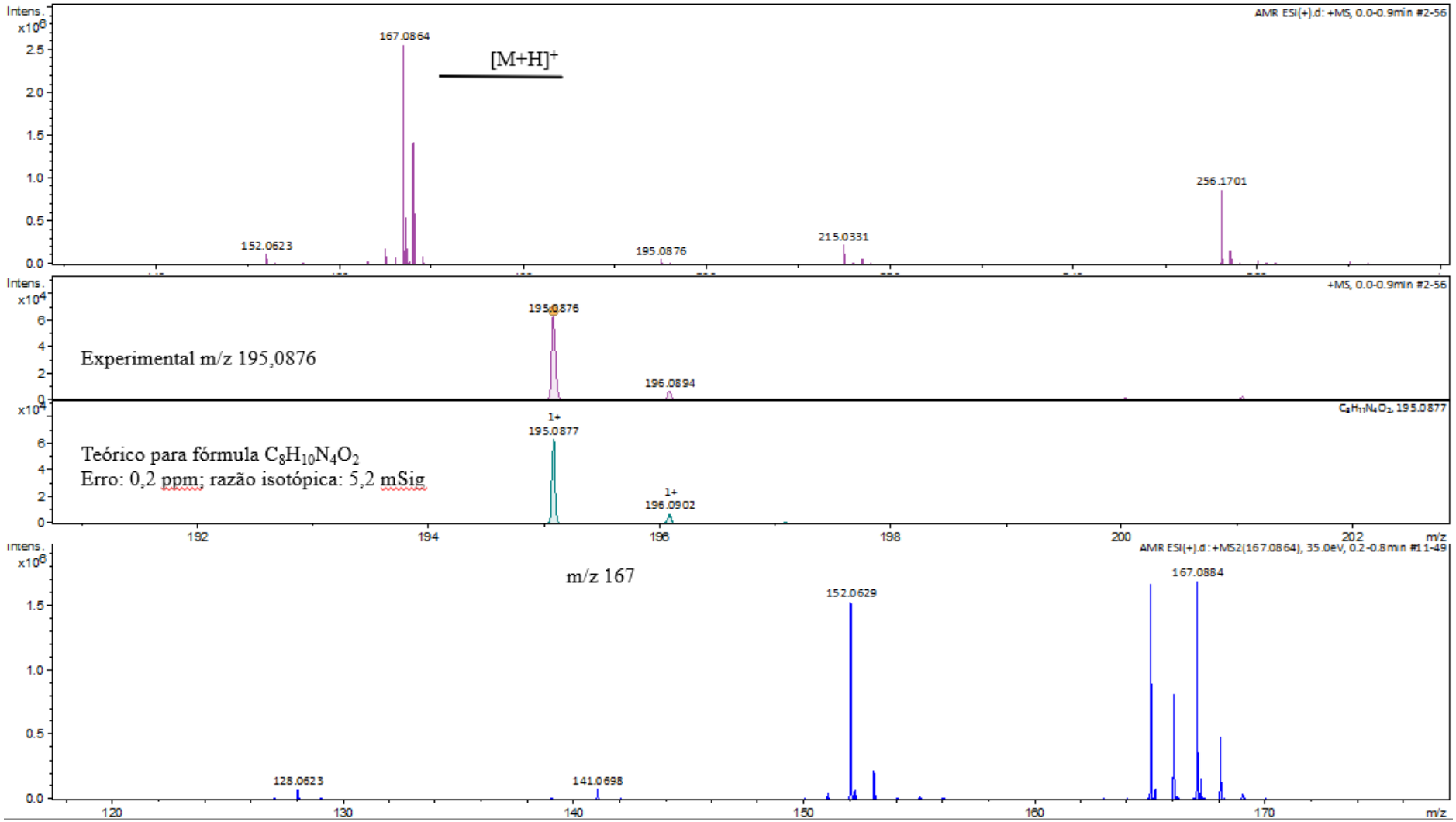
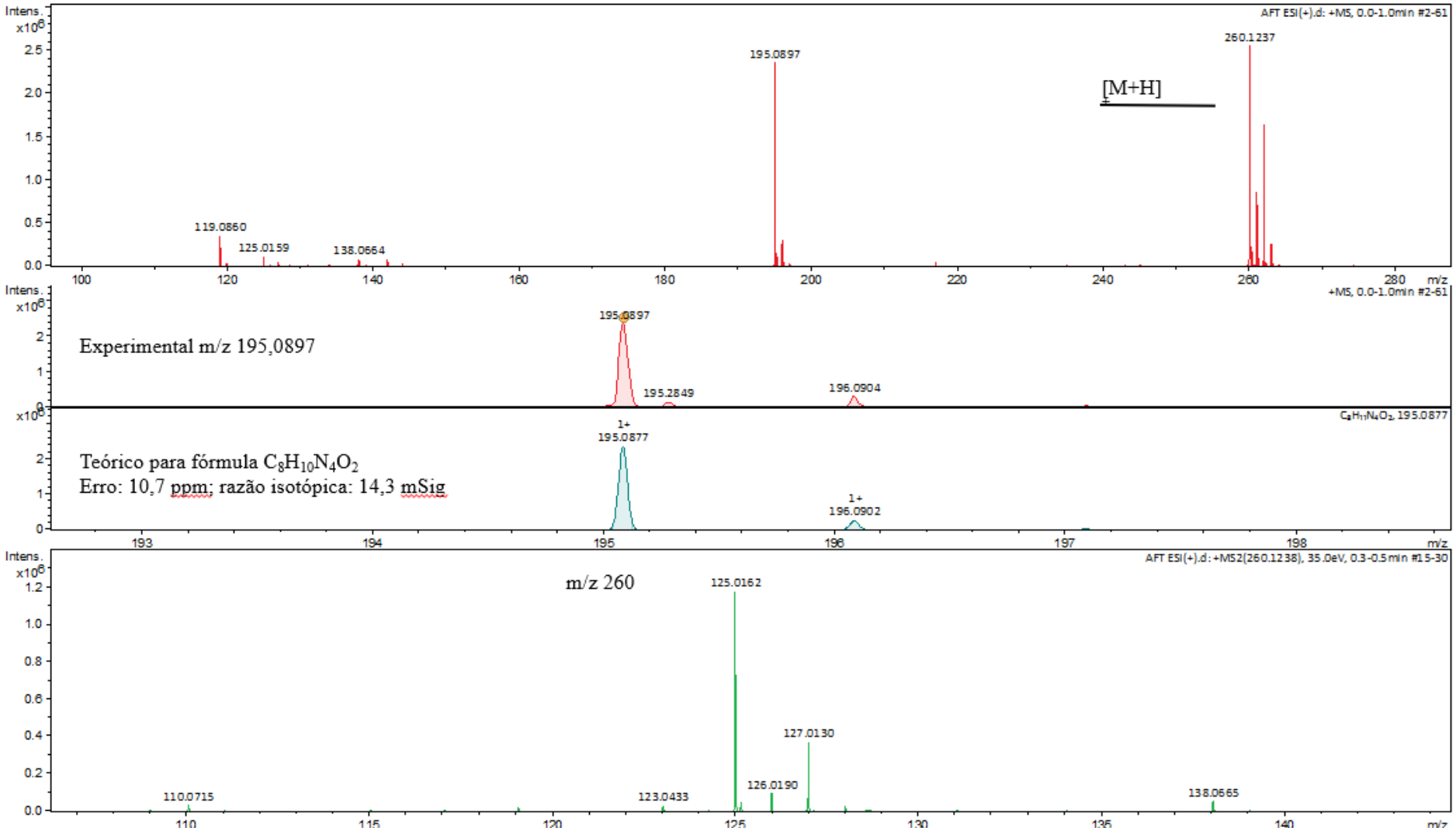


Figura 5 - Espectros de EMAR para a amostra AFT



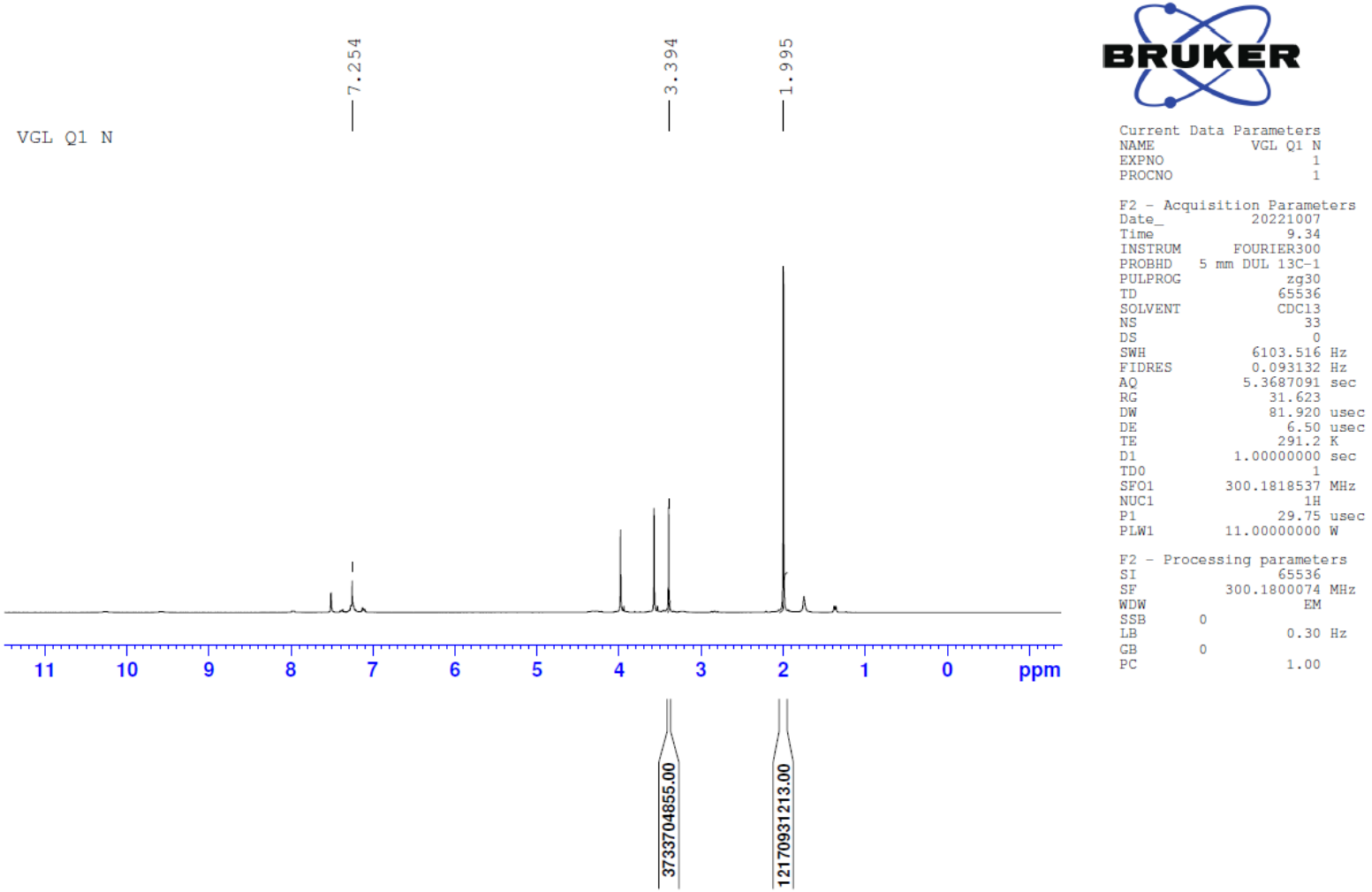
DETERMINAÇÃO DE COMPOSTOS NÃO-ALVO POR HRMS E QUANTIFICAÇÃO DE CAFEÍNA POR RMN EM DROGAS USADAS POR MOTORISTAS PROFISSIONAIS NO BRASIL

TAINARA GUIZOLFI

APÊNDICE B – ESPECTROS DE RESSONÂNCIA DAS AMOSTRAS DE REBITES

ÍNDICE

Figura 1 - Espectros de RMN para a amostra VGL



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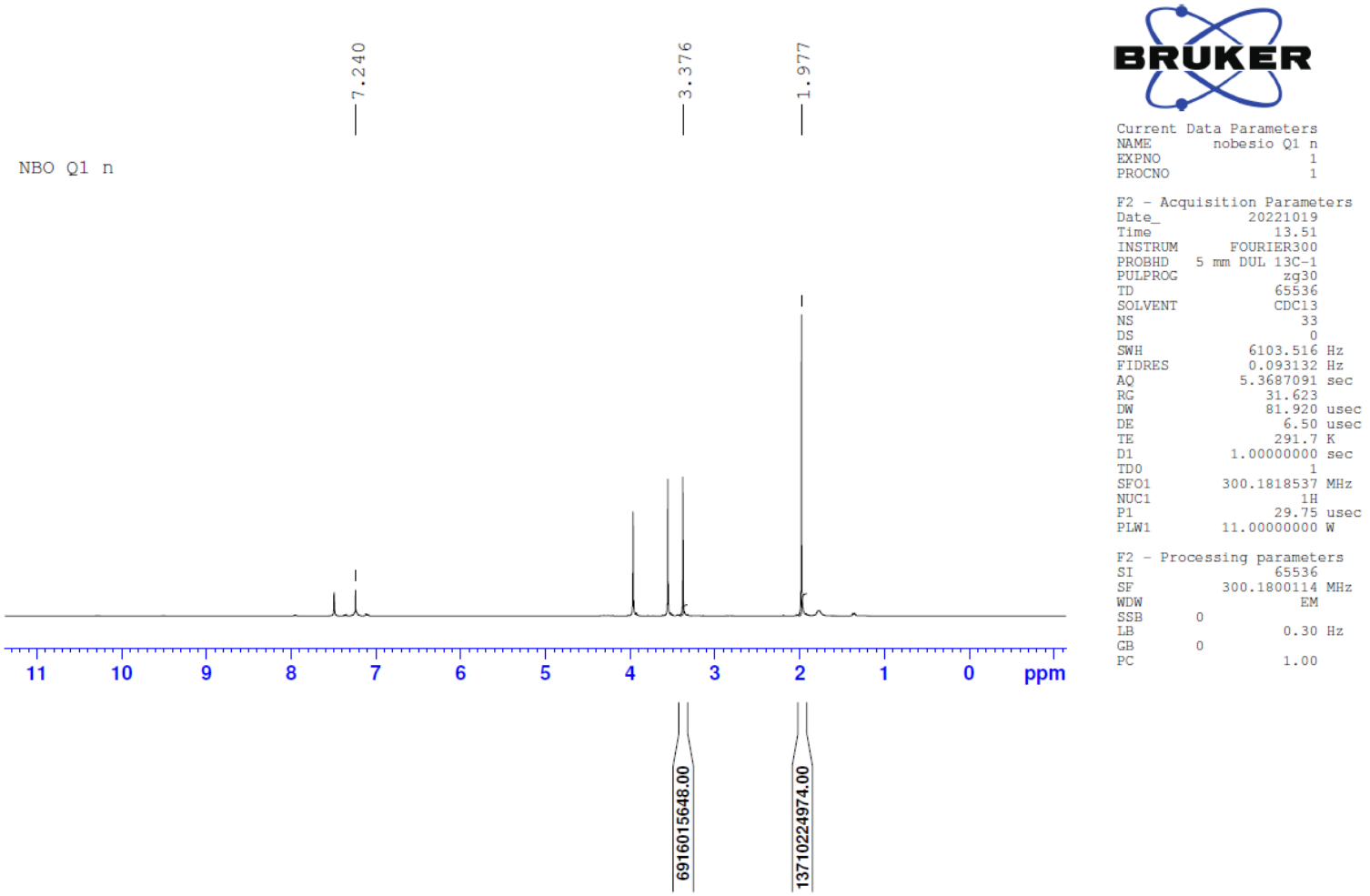

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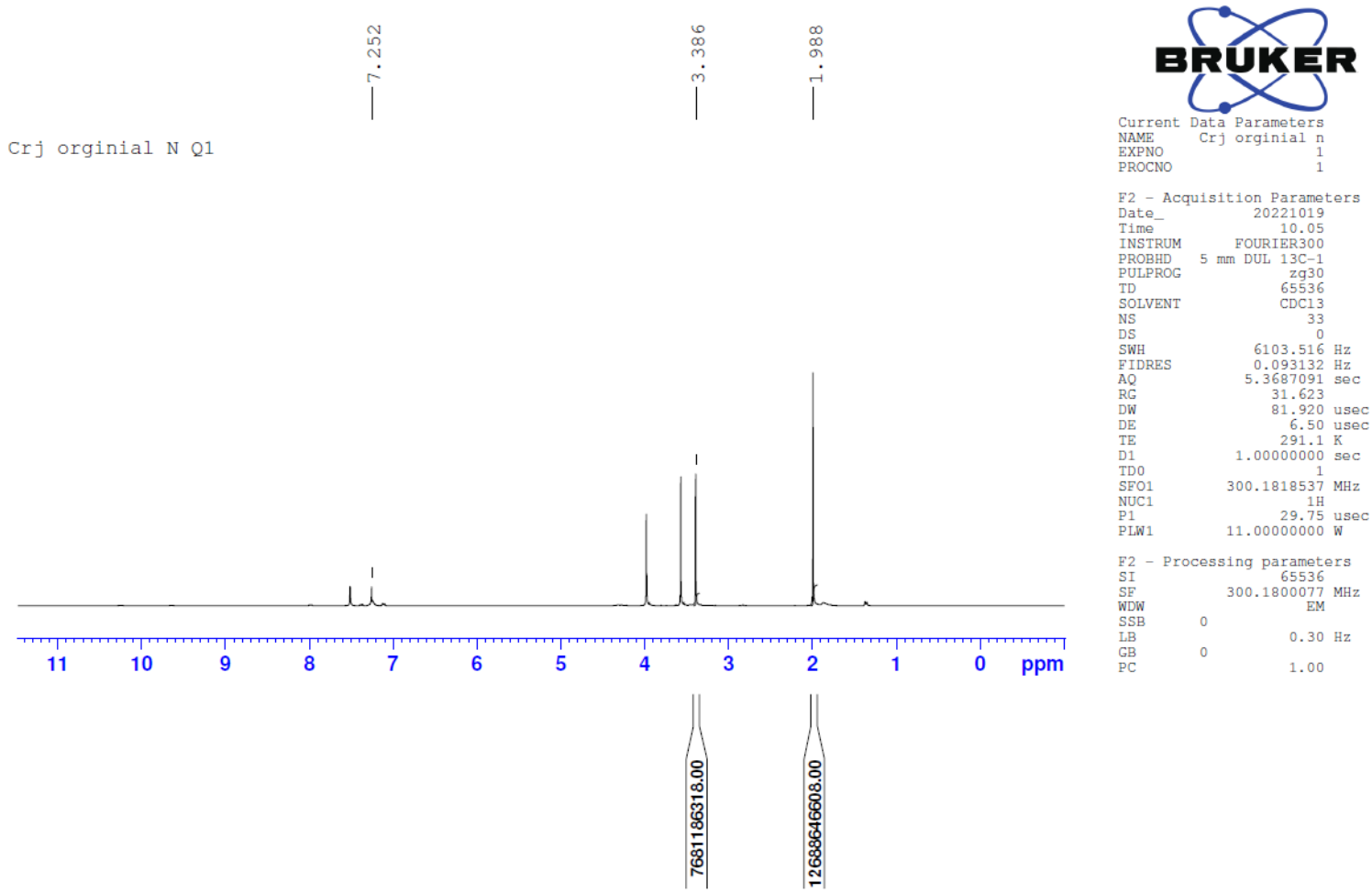

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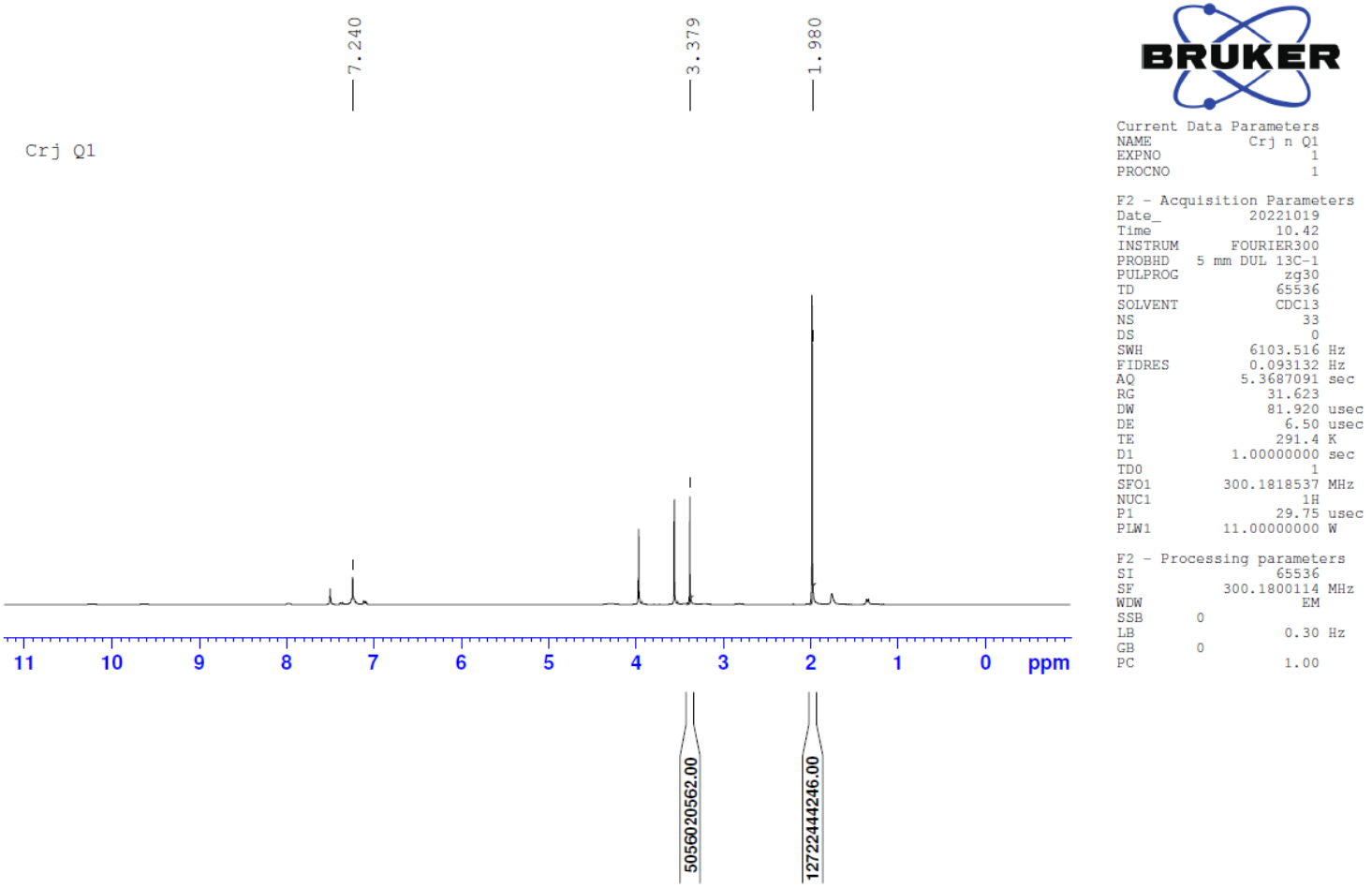


Figura 5 - Espectros de RMN para a amostra AMR

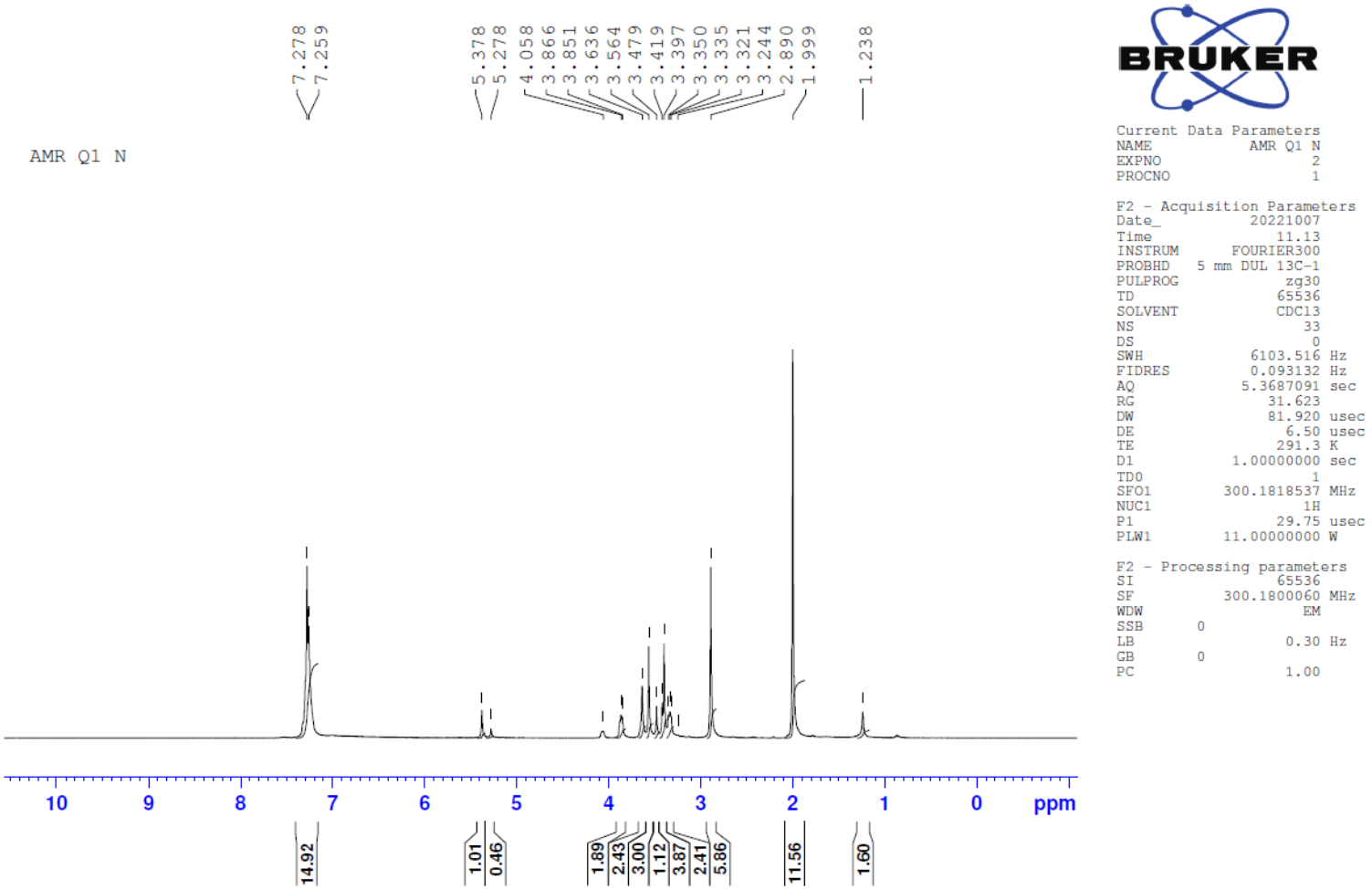
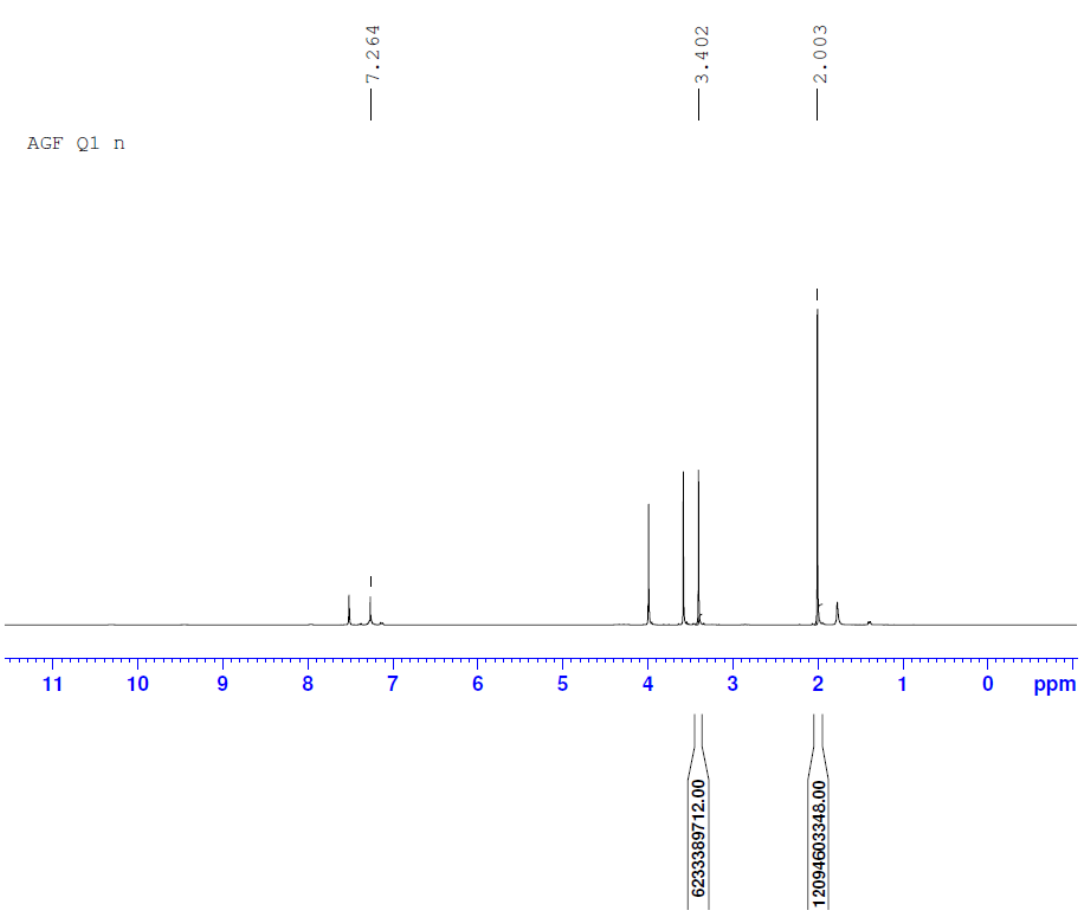


Figura 6 - Espectros de RMN para a amostra AFT



BRUKER

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