UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

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Analysis of different drug extraction methods in biological fluid samples for LC-MS/MS assays: scoping review

Análise de diferentes métodos de extração de drogas em amostras de fluido biológico para ensaios LC-MS/MS: revisão de escopo

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Orientadora: Profa. Dra. Adriana Maria Calvo

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“Talvez não tenha conseguido fazer o melhor, mas lutei para que o melhor fosse feito. Não sou o que deveria ser, mas Graças a Deus, não sou o que era antes”.

Martin Luther King

RESUMO

O propósito deste estudo foi realizar uma investigação sistematizada e análise de diferentes métodos de extração de fármacos, especificamente anti-inflamatórios não-esteróides, em amostras de fluidos biológicos para ensaios em LCMS/MS. A busca foi realizada nas bases de dados PubMed, Scopus (Elsevier), Scientific Electronic Library Online (SciELO), Google Scholar e a Biblioteca Digital de Teses e Dissertações da USP, entre os anos de1999 e 2021, seguindo as diretrizes do guia Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) Checklist. A estratégia de busca foi efetuada com operadores booleanos (“AND” e “OR”), onde inicalmente foram selecionados 248 artigos. Após a verificação por dois revisores independentes, remoção de duplicatas, leitura de títulos, resumos e texto completo, obtivemos 39 publicações que atenderam aos critérios de inclusão e exclusão (previamente estabelecidos). Em 52% dos estudos, os autores optaram pela extração líquido-líquido, enquanto 41% pela extração em fase sólida. 5% empregam métodos de microextração e 2% utilizam técnicas menos convencionais. A extração LLE é a técnica comumente empregada na preparação de amostras e a que apresentou maior seletividade comparado a métodos simples de extração por solvente e a mais antiga das técnicas aplicadas para determinar compostos químicos. Contudo, mesmo que tenha ampla utilização e bom desempenho analítico, a LLE apresenta diversas desvantagens, como necessidade de grandes volumes de amostra e solventes orgânicos. No tempo presente, a LLE é considerada uma técnica cara, demorada, dispendiosa e não atende aos requisitos atuais da química analítica verde, isto é, contrapondo as tendências inovadoras no manuseio de amostras e técnicas de extração mais rápidas, seguras e ecológicas.

Palavras-chave: Cromatografia Líquida, Extração Líquido-Líquido, Espectrometria de Massas, Métodos Analíticos de Preparação de Amostras.

ABSTRACT

Analysis of different drug extraction methods in biological fluid samples for LC-MS/MS assays: scoping review

The purpose of this study was to carry out a systematic investigation and analysis of different drug extraction methods, specifically non-steroidal anti-inflammatory drugs in biological fluid samples, for Liquid Chromatography in Mass Spectrometry assays. The search was carried out in PubMed, Scopus (Elsevier), Scientific Electronic Library Online (SciELO), Google Scholar, and the Digital Library of Theses and Dissertations of the University of São Paulo, between 1999 and 2021, following the guidelines of the Preferred Reporting guide. Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRSMA-ScR) Checklist. The search strategy was carried out with Boolean operators (“AND” and “OR”), where initially 248 articles were selected. After verification by two independent reviewers, removal of duplicates, reading of titles, abstracts, and full text, 39 publications that met the inclusion and exclusion criteria (previously established) were obtained. In 52% of the studies, the authors used the liquid-liquid extraction method, while in 41% the solid-phase extraction method was used. 5% used microextraction methods and 2% used less conventional techniques. Liquid-liquid extraction is the technique commonly used in sample preparation and the one with the highest selectivity, compared to simple solvent extraction methods, and the oldest of the techniques applied to determine chemical compounds. However, even though it has wide use and good analytical performance, liquid-liquid extraction has several disadvantages, such as the need for large volumes of samples and organic solvents. Currently, liquid-liquid extraction is considered an expensive, time-consuming technique and does not meet the current requirements of green analytical chemistry, that is, going against innovative trends in sample handling and faster, safer, and more environmentally friendly extraction techniques.

Keywords: Liquid chromatography, Liquid-liquid extraction, Mass spectrometry, Sample Preparation Methods.

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ABREVIATURA E SIGLAS

AINES	anti-inflamatórios não esteroidais
CG	cromatografia gasosa
DLLME	microextração líquido-líquido dispersiva
DSPE	extração em fase sólida dispersiva
HPLC	cromatografia líquida de alta eficiência
UHPLC/PDA	cromatografia líquida de ultra-alta eficiência - arranjo de fotodiodos
LC	cromatografia líquida
LLE	extração líquido-líquido
MEPS	microextração com sorvente empacotado
MS	espectrometria de massa
MSPE	extração em fase sólida magnética
SBSE	extração sortiva em barra de agitação
SPE	extração em fase sólida
SPME	microextração em fase sólida
UV	ultravioleta

SUMÁRIO

1. INTRODUÇÃO

Os anti-inflamatórios não esteroidais (AINEs) são normalmente a classe de medicamentos, seja sob prescrição ou não, de escolha dos pacientes para controlar sinais e sintomas inflamatórios. Estima-se que mais de 30 bilhões de AINES sejam vendidos por ano (ROLLASON et al., 2014). Esta classe de medicamentos geralmente se mostra efetiva quando empregada no controle da dor, pois sua ação ocorre na inibição da ciclooxigenase. No entanto, o uso de AINEs está também associado a reações adversas graves, principalmente no trato gastrintestinal, no sistema renal e cardiovascular (HARGREAVES; ABBOTT, 2005; ROLLASON et al., 2014).

Atualmente, inúmeros ensaios com tais medicamentos têm sido quantificados pela técnica de cromatografia líquida acoplada á espectrometria de massas (LC-MS/MS), pois a seletividade desse método e a preparação de amostra, em suma, são menos dispendiosas. Estes ensaios, que habitualmente manipulam amostras de sangue, plasma, saliva, urina, entre outros fluídos biológicos, mostram-se superiores quando comparados com imunoensaios ou espectrometria de massa por cromatografia gasosa (GC-MS) (SUHR et al., 2016; WANG et al., 2014).

A Espectrometria de Massas (MS) é a técnica analítica que identifica e quantifica compostos desconhecidos ou materiais conhecidos com sensibilidade em níveis sub-nanomolares. No momento atual, ocorreu um grande crescimento na área de MS e na sua utilização em áreas clínicas. Tal fato se deve ao acoplamento da cromatografia líquida ao MS ou MS/MS, o qual criou a Cromatografia Líquida em Espectrometria de Massas (LC-MS/MS), que expande sua aplicação para maior peso molecular ou moléculas extremamente polares, tornando o LC-MS/MS uma técnica que permite uma rápida determinação de substâncias conhecidas com apenas uma pequena quantidade de material de origem (LEUNG; FONG, 2014).

Nesta técnica, a amostra é primeiramente separada pela cromatografia líquida de alta eficiência (HPLC) e o efluente da coluna cromatográfica é transferido para um espectrômetro de massa. A separação por Cromatografia Líquida (LC) é baseada nas propriedades físicas e químicas dos analitos tais como: hidrofobicidade, tamanho molecular, presença de grupos funcionais, entre outros. Portanto, quando o efluente atinge a fonte de íons no espectrômetro de massas, ocorre a ionização e o espectrômetro de massas produz íons com intensidade de sinal proporcional à quantidade de analito presente na amostra. Nesta técnica hifenizada, o LC resolve

componentes individuais da amostra, enquanto o espectrômetro de massas detecta seletivamente esses componentes (LEUNG; FONG, 2014; VOGESER; SEGER, 2008).

O pré-tratamento da amostra, nesta técnica, compreende etapas dispendiosas e, devido a isso, este foi considerado, por anos, de menor importância. O objetivo da preparação da amostra visa a remoção de componentes da matriz que interferem na separação e/ou detecção e conversão do analito para uma forma e concentração adequada, aumentando assim a sensibilidade. Atualmente, com as melhorias nas colunas de cromatografia e cromatografia líquida, tornar a conversão do analito para uma forma adequada para separação, muitas vezes, faz-se desnecessário (LAAKS; JOCHMANN; SCHMIDT, 2012).

No caso das amostras biológicas, tais como alimentos, urina, plasma, cabelo, saliva, entre outras, seu preparo para as análises LC-MS/MS é um desafio inerente a sua complexidade, pois nem todas as técnicas de preparação podem ser usadas, uma vez que grande parte das amostras clínicas são a base de água ou pertencem ao grupo que normalmente incluem vários componentes que ocupam ou fornecem íons, como hidrogênio, sódio e amônio, causando supressão ou aprimoramento de íons na análise LC-MS/MS, (HE et al., 2014; LAAKS; JOCHMANN; SCHMIDT, 2012; LEUNG; FONG, 2014). Além disso, os fosfolipídios, comuns em amostras clínicas, são bem conhecidos por seu efeito supressor de íons. Portanto, a remoção desses compostos é fundamental para a análise LC-MS/MS (HE et al., 2014; LAAKS; JOCHMANN; SCHMIDT, 2012; LEUNG; FONG, 2014).

Dentre as amostras biológicas, a saliva, por exemplo, tem chamado a atenção como uma nova ferramenta em ensaios clínicos de monitoramento de drogas terapêuticas, pois esta apresenta uma amostragem fácil, não invasiva, sem estresse e repetida em tempo real, enquanto a coleta de sangue ou plasma é indesejável, já que muitas vezes causam hematomas, incômodo ou não são realizadas pelos pacientes por medo, considerando as múltiplas coletas necessárias. A análise da saliva tem um grande potencial para facilitar a pesquisa clínica (OGAWA et al., 2014), contudo, há ainda uma escassez de publicações com este fluido. Neste sentido, neste estudo, foram incluídos na pesquisa bibliográfica todos os fluidos onde houve análise de concentrações dos AINES por LC-MS/MS.

Os meios de extração de fluidos biológicos, comumente empregados, se dividem em duas categorias com diferentes fases de extração: o método que utiliza

solventes como a extração líquido-líquido (LLE) e suas variações, entre outras, e a extração utilizando materiais sorventes como a extração em fase sólida (SPE) (HUANG; LEE, 2012; REZAEE et al., 2006; REZAEE; YAMINI; FARAJI, 2010).

Novas técnicas, como a de microextração, apresentam vantagens sobre as técnicas clássicas (LLE ou SPE), a saber: utilização de menos solventes e número e tamanho mínimo de amostras; redução das etapas de tratamento da amostra; diminuição no consumo de reagentes perigosos e maximização de energia, ampliando a segurança para os operadores e corroborando com o meio ambiente, gerando menos resíduos; aplicações crescentes na etapa de preparação da amostra, antes da determinação cromatográfica de analitos em amostras biológicas complexas (FILIPPOU; BITAS; SAMANIDOU, 2017; LUIZ; MACIEL; LANÇAS, 2015).

Posto isto, técnicas de preparo franqueáveis e mais benéficas ao meio ambiente têm ganho espaço na química analítica e progredido em direção ao desenvolvimento de novos métodos para miniaturização de técnicas clássicas bem estabelecidas (HUANG; LEE, 2012).

A revisão de escopo tem sobressaído na área de síntese de evidências em saúde e propõe a realização do mapeamento da literatura num determinado nicho de interesse, sobretudo quando revisões acerca de um tema ainda não foram publicadas (CORDEIRO; SOARES, 2019). Adequada a tópicos amplos, a revisão de escopo permite reunir vários desenhos de estudos, sendo este fato o que a distingue da revisão sistemática, pois seu objetivo não visa buscar pela melhor evidência sobre uma intervenção ou experiência, mas sim reunir os vários tipos de evidências e mostrar como foram produzidas. E assim como acontece nos estudos primários, é a pergunta que dirige a metodologia de revisão a ser adotada (CORDEIRO; SOARES, 2019; TRICCO et al., 2018).

Desse modo, a revisão de escopo permite tanto um auxílio ao revisor que necessita examinar evidências emergentes, quanto examinar como as pesquisas estão sendo conduzidas em áreas já consolidadas (CORDEIRO; SOARES, 2019; TRICCO et al., 2018).

O propósito deste estudo foi realizar uma investigação sistematizada e análise de diferentes métodos de extração de fármacos, especificamente AINEs, em amostras de fluidos biológicos para ensaios em LCMS/MS. Para tanto, foram consultados artigos e teses indexados e/ou listados nas plataformas PubMed, Scopus (Elsevier), Scientific Electronic Library Online (SciELO), Google Scholar e a Biblioteca Digital de

Teses e Dissertações da USP, entre os anos de1999 e 2021. Sendo assim formulou-se a seguinte questão de pesquisa:

- Qual é a metodologia e/ou técnicas mais utilizadas para a extração de anti-inflamatórios não esteroides em ensaios bioanalíticos utilizando cromatografia líquida de alta eficiência e espectrometria de massa (LC-MS/MS)?

2. ARTIGO

The article presented in this Dissertation was written according to the **Brazilian Journal of Pharmaceutical Sciences** instructions and guidelines for article submission.

2.1 Artigo 1

Analysis of different drug extraction methods in biological fluid samples for LC-MS/MS assays: Scoping review

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Abstract

The purpose of this study was to carry out a systematic investigation and analysis of different drug extraction methods, specifically non-steroidal anti-inflammatory drugs in biological fluid samples, for Liquid Chromatography in Mass Spectrometry assays. Thirty-nine publications and their results, published in English, from 1999 to 2021 were included in the study. In 52% of the studies, the authors used the liquid-liquid extraction method, while in 41% the solid-phase extraction method was used. 5% used microextraction methods and 2% used less conventional techniques. Liquid-liquid extraction is the technique commonly used in sample preparation and the one with the highest selectivity, compared to simple solvent extraction methods, and the oldest of the techniques applied to determine chemical compounds. However, even though it has wide use and good analytical performance, liquid-liquid extraction has several disadvantages, such as the need for large volumes of samples and organic solvents. Currently, liquid-liquid extraction is considered an expensive, time-consuming technique and does not meet the current requirements of green analytical chemistry, that is, going against innovative trends in sample handling and faster, safer, and more environmentally friendly extraction techniques.

Keywords: Liquid chromatography, Liquid-liquid extraction, Mass spectrometry, Sample Preparation Methods

Running title: Analysis of different drug extraction methods for LC-MS/MS: Scoping review

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1. Introduction

Mass Spectrometry (MS) is an analytical technique that identifies and quantifies unknown compounds or known materials with sensitivity at sub-nanomolar levels. In its beginning, during the 1950s, it was often used in conjunction with gas chromatography (GC), but its use was limited to the detection of small volatile molecules such as fatty acids, organic acids, amino acids, monosaccharides, prostaglandins, bile acids and steroids (Leung, Fong, 2014). In recent years, there has been a growth in the MS area and in its use in clinical areas. Liquid chromatography (LC) has been coupled with MS or MS/MS creating Liquid Chromatography in Mass Spectrometry (LC-MS/MS), which expanded its application to higher molecular weight or extremely polar molecules such as tetrodotoxin in human plasma and urine (Leung, Fong, 2014).

LC-MS/MS is a hyphenated technique, that is, it is a technique that allows a rapid determination of known substances with only a small amount of source material. The sample is first separated by High-performance liquid chromatography (HPLC) and the effluent from the chromatography column is transferred to a mass spectrometer. LC separation is based on the physical and chemical properties of the analytes such as hydrophobicity, molecular size, presence of functional groups, etc. Therefore, when the effluent reaches the ion source in the mass spectrometer, ionization occurs and the mass spectrometer produces ions with signal intensity proportional to the amount of analyte present in the sample. In this hyphenated technique, the LC solves individual components of the sample in time, while the mass spectrometer selectively detects these components (Leung, Fong, 2014; Vogeser, Seger, 2008).

The manipulation or pre-treatment of the sample comprises one of the most time-consuming and laborious steps of the analytical procedures and for years it was considered of minor importance. The purpose of sample preparation is to remove matrix components that interfere with separation and/or detection and conversion of the analyte to a suitable form and concentration, increasing sensitivity. Today, with the improvements in chromatography and liquid chromatography columns, making the conversion of the analyte to a form suitable for separation is often unnecessary(Laaks, Jochmann, Schmidt, 2012).

The peculiar nature of biological samples makes their preparation for LC-MS/MS analysis a challenge in itself. Most clinical samples are aqueous (single

matrices), but not all preparation techniques can be used for water-based samples, or belong to the group that typically includes multiple components that occupy or supply ions, such as hydrogen, sodium, and ammonium, causing ion suppression or enhancement in LC-MS/MS analysis (He *et al*., 2014; Laaks, Jochmann, Schmidt, 2012; Leung, Fong, 2014). Salts and small hydrophilic molecules can have this effect, while compounds that affect droplet formation by acting as surfactant compounds in the matrix, and also affect ionization efficiency and result in ion suppression or enhancement. Finally, phospholipids, common in clinical specimens, are well known for their ion-suppressing effect. Therefore, the removal of these compounds is critical for LC-MS/MS analysis. Examples of complex matrices are food, urine, plasma, and hair, among others (He *et al*., 2014; Laaks, Jochmann, Schmidt, 2012; Leung, Fong, 2014).

In our study, we focused on the use of saliva as the analyzed biological matrix but also included other body fluids such as plasma, whole blood, and cerebrospinal fluid, among others. Saliva has gained attention as a new tool in clinical trials and monitoring of therapeutic drugs because it offers easy, non-invasive, stress-free, and real-time repeatable sampling, while blood or plasma collection is undesirable, as sometimes causes bruising, discomfort, or even are not performed by patients for fear, considering the multiple collections necessary. Therefore, analysis of analytes in saliva has great potential to make clinical research easier (Ogawa *et al*., 2014).

1.1 Extraction techniques found in the literature

Affordable, effective, and more environmentally friendly preparation techniques have gained space in analytical chemistry and progressed towards the development of new methods aimed at miniaturization of well-established classical techniques (Huang, Lee, 2012). The means commonly used are divided into two categories, with different extraction phases, being the method that uses solvents such as LLE, dispersive liquid-liquid microextraction (DLLME) and its variations, among others, and extraction using sorbent materials such as solid-phase extraction (SPE), stir bar sorption extraction (SBSE) and solid-phase microextraction (SPME) (Huang, Lee, 2012; Rezaee *et al*., 2010, 2006).

1.1.1 Methods that use solvents

I. **Liquid-Liquid Extraction (LLE)** is a typical technique used for the preparation of biological samples of an aqueous nature. Practically, an equivalent or more extraction solvent is used to extract all analytes from the original samples. After extraction, the solvent is evaporated and reconstituted. LLE has a number of disadvantages that restrict its use in laboratories, including limited selectivity, difficulty in automation, and inability to handle emulsions. Solvents used are normally non-polar organic solvents. When using them, hydrophobic analytes are extracted into the organic layer, but other non-polar components (e.g. serum lipids) are often co-extracted (Gopinath *et al*., 2013; Leung, Fong, 2014; Rezaee *et al*., 2006).

II. **Dispersive Liquid-Liquid Microextraction (DLLME)**

It consists of the distribution balance of the analyte between the donor (sample) and acceptor (organic solvent) phases and is ideal for the extraction of compounds with moderate and high lipophilic properties or that may have their distribution coefficient altered by pH control (acid or basic analytes) (Ojha *et al*., 2009; Rezaee *et al*., 2006).

III. **Parallel artificial liquid membrane extraction (PALME)**

It was introduced in 2013 as a new extraction technique. The technique is an extension of liquid-phase microextraction (LPME) in a 96-well format. Two 96-well plates, a donor plate and a recipient plate, are used to perform the extractions. PALME is performed with commercially available 96-well plates and the extraction procedure offers a simple workflow. Its automation potential is high in addition to offering a high degree of sample cleanliness and can be considered a contribution to “green chemistry”, as the use of organic solvent per sample is low (3–5 µL) (Ask *et al*., 2018).

IV. **Magnetic solvent bar liquid-phase microextraction (MSB-LPME)**

In the MSB-LPME method, a stainless steel rod is inserted into the hollow fiber lumen and an organic solvent, which was not only used as an extraction unit but also acted as a magnetic stirrer and a magnetic separator, immobilizes the fiber pores to form an MSB. In the LPME, in addition to immobilizing the extraction solvent, the hollow fiber also has a cleaning capacity due to its microporous structure in the membrane wall. However, the filtering effect of

hollow fiber is insufficient for the elimination of large molecules (e.g. proteins) in blood samples (Li *et al*., 2020).

1.1.2 Methods that use sorbents

As an alternative technique, and the one most commonly used in clinical laboratories, we also find SPE. The devices consist of small columns that contain cartridges with appropriate packaging and the choice of sorbents will depend on the analyte to be extracted. The sorbent is isolated and a specific organic solvent is used to elute the analyte. Among the advantages of SPE are selectivity, flexibility, and high automation potential. SPE products are available in various shapes, sizes, and separation mechanisms such as polar, non-polar, ion Exchange, etc. The 96-well plate format is suitable for automation and is typically employed in high sample throughput clinical laboratories (Filippou *et al*., 2017; Leung, Fong, 2014). It is a common sampling technique in several areas, including pharmaceutical, food, and clinical, among others (Huck, Bonn, 2000; Poole, 2003).

I. Stir bar sorptive extraction (SBSE)

The technique is based on the partition of the analyte between the aqueous phase and the extractor phase (sorbent film on the magnetic bar), with an equilibrium between them. A stir bar is coated with sorbent materials in large quantities compared to SPME fiber. Thus, the absorption capacity increases and the recovery values as well, contributing so that the chemical properties of the surface area are not lost, resulting in innovations and obtaining more efficient and faster sample preparations (Neng *et al*., 2010).

II. Solid-phase microextraction (SPME)

This technique consists of placing the sorbent, in this case, the fiber (extractor phase), in contact with the matrix, for a determined time, so that the analyte adsorbs on the fiber surface. These analytes are desorbed by heat treatment or using a device known as a Holder. Basically, it is the miniaturization of SPE and one of its main features is the extraction of volatile or semi-volatile compounds (Aziz-Zanjani, 2014; Mehdinia, Aziz-Zanjani, 2013).

III. Solid-phase microextraction IN-TUBE SPE

It is an efficient preparation technique that uses an open fused silica capillary tube, and an internally coated GC column with a stationary phase can be used as an SPME device. It was developed with the purpose of overcoming the disadvantages of

conventional SPME, such as low sorption capacity and high fragility, with the advantage of direct extraction of the analytes of interest from the aqueous matrix and their concentration in the stationary phase that covers the capillary internally. In this system, a capillary column is used for solid-phase extraction, coupling it in line with the HPLC system (in-tube SPME-LC) allowing, thus, the automation of the extraction process, greater precision of the analytical method. and shorter analysis time (Toffoli, Lancas, 2015).

IV. Microextraction by a packed sorbent (MEPS)

MEPS can be employed in two basic configurations: the sorbent in the syringe configuration and the cylinder insertion and needle configuration (BIN). A typical application of MEPS includes sorbent conditioning, sample loading, washing, and analyte elution. It is a fast, simple, and inexpensive bioanalytical technique with reduced sample, solvent, and sorbent requirements. MEPS can be semi or fully automated, with online coupling capacity and autosampler support for LC and GC analysis. As a result, MEPS could replace SPE cartridges in any of the existing SPE methods (Altun, Abdel-Rehim, 2008; Filippou *et al*., 2017; Toffoli, Lancas, 2015).

Microextraction techniques have advantages over classical techniques (LLE or SPE), such as using fewer solvents and minimum number and size of samples, reduction of sample treatment steps, reduction in the consumption of hazardous reagents, and energy maximization. This enhances safety for operators and supports the environment, generating less waste, presenting increasing applications in the sample preparation step prior to chromatographic determination of analytes in complex biological samples such as saliva, blood, plasma, serum, urine, hair, cerebrospinal fluid, etc (Filippou *et al*., 2017; Toffoli, Lancas, 2015).

The purpose of this scoping review is to structure a survey carried out in this area, as well as to identify any gaps in existing knowledge. Therefore, the following research question was formulated:

- What is the methodology and/or techniques most used for the extraction of non-steroidal anti-inflammatory drugs in bioanalytical assays using high-performance liquid chromatography and mass spectrometry (LC-MS/MS)?

2. Material and methods

This study was carried out to investigate and analyze different methods of drug extraction in biological fluid samples for LC-MS/MS assays, following the guidelines of the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA- ScR) Checklist and final protocol was prospectively registered with the Open Science Framework on April 24, 2022 (https://osf.io/nmjpy/) (Tricco *et al*., 2018).

Data were collected in PubMed, Lilacs, Embase, Scopus, and Web of Science electronic databases. The search strategy used Boolean operators (AND and OR): ("Analytic sample preparation methods" OR "Extraction, Liquid-Liquid" OR "Liquid Liquid Extraction" OR "Extraction, Liquid Phase" OR "Liquid Phase Microextraction" OR " Solid phase extraction" OR "Extraction, solid phase" OR "Solid phase microextraction") AND ("Anti-Inflammatory Agents, Non-Steroidal" OR "Non-Steroidal Anti-Inflammatory Agents" OR "NSAID" OR "Aspirin-Like Agents" ") AND ("Saliv*" OR "Blood" OR "Plasma" OR "Blood plasma" OR "Plasma, blood" OR "Serum" OR "Blood serum") AND ("Mass Spectrometry" OR "LCMS" OR "LC /MS"). End-Note reference manager was used to save search records and eliminate duplicate references.

I. The selection of studies was carried out by two reviewers independently so that, in the first step, titles and abstracts were read and, in the second step, the full texts were read to filter only those that were, in fact, compatible with the eligibility criterion. The following inclusion criteria were applied:

II. Studies with NSAID;

III. Studies with LC-MS/MS;

IV. Studies in English;

V. Studies that presented the analytical methodology well described;

VI. Studies covering extraction methods targeting well-established classical techniques and their miniaturization;

Exclusion Criteria:

I. Studies with drugs other than NSAID;

II. Studies with gas chromatography and variations;

III. Studies carried out with animals;

IV. Literature reviews;

V. Not published in English:

VI. Studies that did not allow access to the full content;

When reading the full text, the following data were provided: first author, year and country of origin of the study; type of study, analyte, equipment used for analysis, type of ionization, sample preparation method, and the matrix used. Articles that contained these data were included in the review. During all stages of study selection, a third reviewer was helping to solve discrepancies.

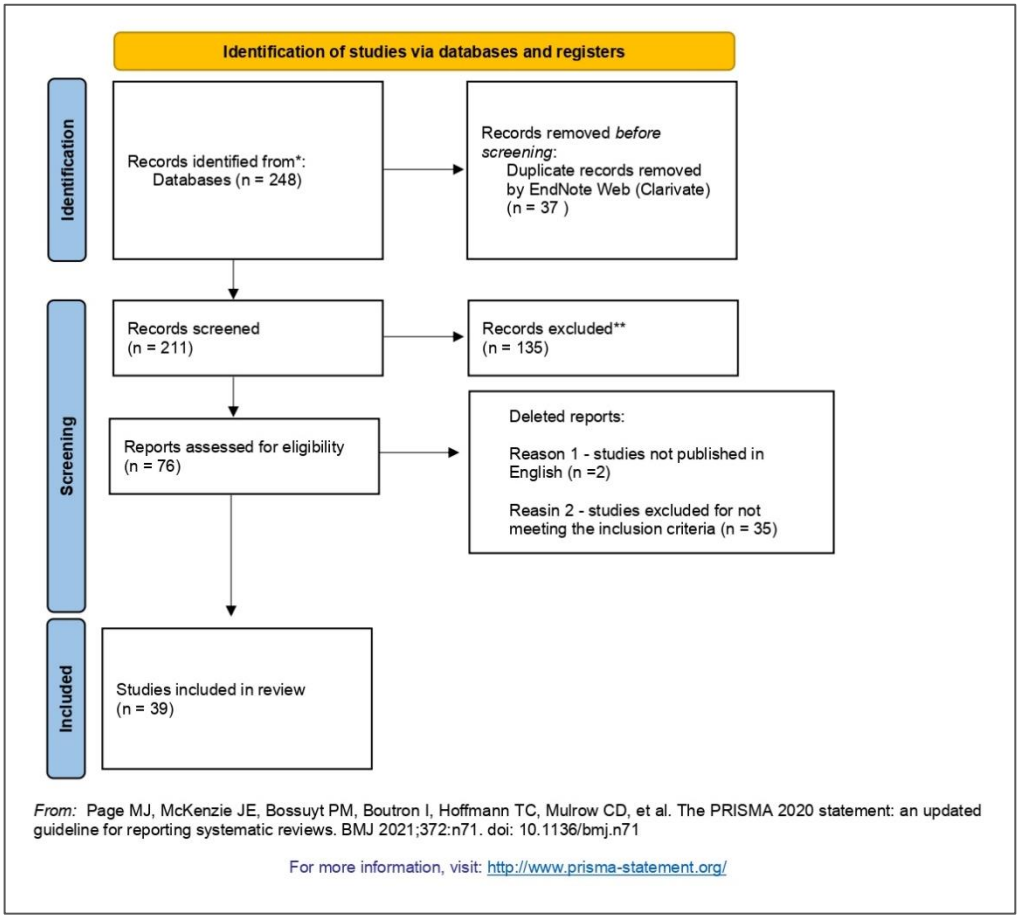
3. Results

3.1 Selection of studies

The search in the database resulted in 248 studies. After removing the duplicates, 211 remained. After reviewing the title and abstract, only 79 studies were evaluated and passed to the next phase, which comprised the complete reading of the article. 39 publications were considered eligible for this review (Figure 1).

Figure 1. PRISMA flow diagram

Figure 1. The PRISMA 2020 flowchart for new systematic reviews only included database and registry searches



3.2 Study characteristics

The vast majority of the studies found were bioanalytical trials or bioequivalence studies, published in English from 1999 to 2021, carried out in Norway, Japan, Germany, India, Brazil, United States, Korea, China, United, Kingdom, Macedonia, Egypt, and Australia. A description and classification of the studies found are presented in Table 1.

Table1. Systematic investigation and analysis of different drug extraction methods

AUTHOR/ YEAR/ COUNTRY	TYPE OF STUDY	ANALYTE	EQUIPMENT	IONIZATION	SAMPLE METHOD PREPARATION	MATRIX
(Ask et al., 2018), Norway	Microsampling Assay	Amitriptyline, quetiapine, ketoprofen, fenoprofen, flurbiprofen and ibuprofen	UHPLC-MS/MS Thermo Scientific LTQ XL Linear Ion Trap (Thermo Scientific, Califónia, USA)	Electrospray (ESI)	Liquid-liquid Extraction (LLE) Parallel Artificial Liquid Membrane Extraction (PALME)	Whole Blood
(Banda *et al*., 2016), India	Bioanalytical Assay	Olsalazine Sodium	UHPLC (Shimadzu, Kyoto, Japan) MS/MS Triple quadrupole API-6500 (MDS Sciex, Ontário, Canada)	Turbo Ion spray	LLE	Human Plasma
(Barrientos-Astigarraga *et al*., 2001), Brazil	Bioequivalence Study	Nimesulide	LCMS/MS Micromass Quattro II (Waters Corporation / Micromass Uk Ltd, Manchester, UK)	ESI	LLE	Human Plasma
(Bharwad *et a*l., 2020), India	Pharmacokinetic Study	Fenoprofen	UHPLC Waters Acquity (Waters Corporation, Massachusetts, USA) MS/MS Quattro Premier XE™ (Waters Micro Mass Technologies, Massachusetts, USA)	ESI	Solid Phase Extraction (SPE) - Orochem DVB-LP	Human Plasma
(Bolani *et al*., 2021), Brazil	Bioanalytical Assay	Piroxicam	LCMS/MS Triple quadrupole Quattro Micro (Waters Corporation / Micromass Uk Ltd, Manchester, UK)	ESI	LLE	Saliva
(Bonato *et al*., 2003), Brazil	Enantioselective Analysis	Ibuprofen	HPLC (Shimadzu, Kyoto, Japan) MS/MS Triple quadrupole Quattro Micro (Waters Corporation / Micromass Uk Ltd, Manchester, UK)	ESI	LLE	Human Plasma
(Bräutigam *et al*., 2003), Germany	Bioanalytical Assay	Etoricoxib	LC Degasser Jasco DG 1580-53 (Gross-Umstadt, Germany) MS/MS Triple quadrupole API 3000 (Applied Biosystems, Langen, Germany)	ESI	SPE - Oasis HLB	Human Plasma
(Brêtas *et al*., 2016), Brazil	Bioanalytical Assay	Naproxene e Sumatriptan	LC-ESI-MS/MS Waters System (Waters Corporation, Massachusetts, USA) MS/MS Quattro LC - triple quadrupole (Waters Corporation, Massachusetts, USA)	ESI	LLE	Human Plasma
(Calvo *et al*., 2016), Brazil	Bioanalytical Assay	Piroxicam e 5'-hidroxypiroxicam	LCMS/MS Triple quadrupole Quatto Micro (Micromass UK Ltd, Manchester, UK)	ESI	LLE	Human Plasma and Saliva
(Dionísio *et al*., 2020), Brazil	Bioanalytical Assay	Naproxen	LCMS/MS quadrupole 8040 (Shimadzu, Kyoto, Japan)	ESI	LLE	Saliva
(Dongari *et al*., 2014), USA	Bioanalytical Assay	Celecoxib	LC–ESI–TOF–MS HPLC Agilent 1100 Series with a Agilent G1969 TOF/MS System (Agilent, Califórnia, EUA)	ESI	SPE - Bond Elute C 18	Human Plasma
(Dubey *et al*., 2019), India	Bioanalytical Assay	Celecoxib	LC-10 (Shimadzu, Kyoto, Japan) MS/MS API 3200 (MDS Sciex, Ontario, Canada)	Turbo Ion spray	LLE	Human Plasma
(Eichhold *et al*., 2000), USA	Bioanalytical Assay	(R)- e (S) – Cetoprofen	HPLC modular Gilson (Gilson Inc, Wisconsin, USA) MS/MS PerkinElmer API III $^{+}$ (MDS Sciex, Ontario, Canada)	ESI	SPE - Oasis HLB	Human Plasma

(Gopinath *et al*., 2013), India	Bioanalytical Assay	Naproxen e Esomeprazole	LCMS/MS Agilent Technologies series 1200 triple quadrupole Agilent 6460 (Agilent Technologies, Germany)	ESI	SPE - Oasis HLB	Human Plasma
(Halder *et al*., 2019), India	Bioequivalence Study	Nimesulide e 4-hidroxynimesulide	LCMS/MS API 2000 MS/MS Tandem triple quadrupole (MDS Sciex, Ontario, Canada)	ESI	LLE	Human Plasma
(Hoke *et al*., 2000), USA	Bioanalytical Assay	Cetoprofen	LCMS/MS PerkinElmer API III + (MDS Sciex, Ontario, Canada) IMPROVED FLUIDITY LIQUID CHROMATOGRAPHY (pcSFC-MS/MS) Gilson (Gilson Inc, Wisconsin, USA)	Turbo Ion spray/ ESI	SPE - Oasis HLB	Human Plasma
(Lee *et al*., 2006), Korea	Pharmacokinetic Study	Zaltoprofen	HPLC Waters 2795 MS/MS Triple quadrupole Waters Micromass Quattro Premier (Waters Corporation/ Micromass Uk Ltd, Watford, UK)	ESI	LLE	Human Plasma
(Lee *et al*., 2008), Korea	Bioanalytical Assay	Etodolac	HPLC Waters 2795 MS/MS Triple quadrupole Waters Micromass Quattro Premier (Waters Corporation/ Micromass Uk Ltd, Watford, UK)	ESI	LLE	Human Plasma
(Lee *et al*., 2014), Korea	Bioanalytical Assay	Flurbiprofen	HPLC Agilent 1200 series (Agilent Technologies Inc, California, USA) MS/MS API 3200 (MDS Sciex, Ontario, Canada)	ESI	LLE	Human Plasma
(Li *et al*., 2020), China	Bioanalytical Assay	Cetoprofen, Naproxen, Indomethacin e Diclofenac	HPLC 20A (Shimadzu, Kyoto, Japan) MS/MS Triple quadrupole 4000 QTrap (AB Sciex, Washington, USA)	ESI	Liquid phase microextraction based on supramolecular magnetic solvent HFIP-alkanol with solvent bar (MSB-LPME based on HFIP-alkanol SUPRAS)	Human Serum
(Mahadik *et al*., 2012), India	Bioanalytical Assay	Mefenamic Acid	LCMS/MS PerkinElmer API-3000 (MDS Sciex, EUA) coupled to high performance liquid chromatography (Shimadzu, Kyoto, Japão)	Atmospheric pressure chemical ionization (APCI)	LLE	Human Plasma
(Mohammed *et al*., 2013), United Kingdom	Microsampling Assay	Ketorolac	HPLC-MS/MS TSQ Quantum Discovery Max triple quadripole (Thermo Scientific, USA)	ESI	LLE	Human Plasma
(Nakov *et al*., 2015), Macedonia	Bioanalytical Assay	Ibuprofen	HPLC-MS/MS TSQ Quantum Discovery Max triple quadripole (Thermo Scientific, USA)	ESI	LLE / SPE	Human Plasma
(Nakov *et al*., 2016), Macedonia	Bioanalytical Assay	Ibuprofen	HPLC-MS/MS TSQ Quantum Discovery Max triple quadripole (Thermo Scientific, USA)	ESI	LLE	Human Plasma
(Ojha *et al*., 2009), India	Bioanalytical Method Validation	4-methylaminoantipyrine - dipyrone active metabolite	LC - Atmospheric pressure ionization (Ion Spray) MS Simple Quadrupole (PerkinElmer MDS Sciex, USA)	APCI	LLE	Human Plasma
(Park *et al*., 2012), Korea	Bioanalytical Assay	Celecoxib	HPLC Agilent 1100 (Agilent, USA) MS/MS Triple quadrupole API-2000 (MDS Sciex, Ontario, Canada)	ESI	LLE	Human Plasma

(Patel *et al*., 2008), India	Bioanalytical Assay	6-methoxy-2-naphthylacetic acid - nabumetone active metabolite	LCMS/MS Triple quadrupole API-3000 (Shimadzu, Kyoto, Japan)	Turbo Ion spray	SPE - Oasis HLB Cartridges	Human Plasma
(Patel *et al*., 2012), India	Bioanalytical Assay	Sumatriptan and Naproxen	UPLC Waters Acquity System and a triple quadrupole Waters Quattro Premier XE (Waters Corporation, Massachusetts, USA)	ESI	SPE - Phenomenex Strata-X Cartridges	Human Plasma
(Patel *et al*., 2013), India	Bioanalytical Assay	Diflunisal - salicylic acid difluorophenyl derivative	LCMS/MS Triple quadrupole API-3000 (Shimadzu, Kyoto, Japan)	ESI	SPE - Oasis HLB Cartridges	Human Plasma
(Scott *et al*., 1999), United Kingdom	Bioanalytical Assay	Green ford-ware Cocktail (Diclofenac)	LCMS/MS 200 series triple quadrupole API-365 (PerkinElmer MDS Sciex, Onario, Canada)	Turbo Ion spray/ ESI	- 96 extraction wells HLB SPE block - Automated Extraction	Human Plasma and Urine
(Shinde *et al*., 2012), Korea	Bioanalytical Assay	Aspirine	HPLC Agilent 1200 series (Applied Biosystems, California, USA) MS/MS QTrap 5500 (Applied Biosystems, California, USA)	ESI	SPE - Discovery DSC-C8 cartridges	Human Plasma
Shirako, 2013, Japan (Shirako *et al*., 2013)	Bioanalytical Assay	Ampiroxicam, tenoxicam, piroxicam, meloxicam and lornoxicam	LCMS/MS API-4000 (AB Sciex, Massachusetts, USA)	ESI	MAX - SPE - Oasis cartridges column	Human Plasma
(Suenami *et al*., 2006), Japan	Bioanalytical Assay	Acetaminofen, aspirine, loxoprofen, cetoprofen, acemetacin, oxaprozin, fenoprofen, flurbiprofen, indomethacin, diclofenac, ibuprofen, henylbutazone, flufenamic acid, mefenamic acid, tolfenamic acid and naproxen	HPLC Alliance 2690 coupled to MS/MS Quadrupole Micromass ZMD (Waters Corporation, Massachusetts, USA)	ESI	SPE - Oasis HLB cartridges	Human Plasma
(Sultan *et al*., 2005), Egypt	Bioanalytical Assay	Salicin, salicylic acid, tenoxicam, ketorolac, piroxicam, tolmetin, naproxen, flurbiprofen, diclofenac and ibuprofen	HPLC 616 model (Waters Corporation, Massachusetts, USA) coupled to a MS/MS Finnigan-MAT TSQ triple quadrupole (Thermo Finnigan, California, USA)	APCI	LLE / SPE - copolymer-based cartridges (poli(N-vinilimidazol-co-divinilbenzeno))	Human Plasma and Whole Blood
(Sun *et al*., 2016), China	Bioanalytical Assay	Nimesulide	LC (Shimadzu, Kyoto, Japan) coupled to a MS/MS QTrap5500 (Applied Biosystems, California, USA)	ESI	LLE	Human Plasma
(Taylor *et al*., 1998), Australia	Bioanalytical Assay	Indometacin	HPLC (Waters Corporation, Massachusetts, USA) coupled to a MS/MS quadruple API III (PerkinElmer MDS Sciex, Ontario, Canada)	ESI	SPE	Human Plasma
(Werner *et al*., 2002), Germany	Bioanalytical Assay	Celecoxib	HPLC (Jasco, Groß-Umstadt, Germany) MS/MS Trap Finnigan MAT LCQ (Thermoquest, Egelsbach, Germany)	APCI	LLE	Human Plasma
(Yu *et al*., 2012), China	Bioanalytical Assay	Cetoprofen, fenbufen and ibuprofen	LC-MS-2010EV HPLC-ESI/MS (Shimadzu, Kioto, Japan)	ESI	In-tube solid phase Microextraction	Human Plasma and Environmental Water
(Zhang *et al*., 2003), USA	Bioanalytical Assay	Valdecoxib	HPLC Agilent 1050 (Agilent, California, USA) MS/MS Quadrupole PerkinElmer Sciex API-III-Plus (Ontario, Canada)	ESI	Autmated system SPE RapidTrace™ - Bond Elut cartridges	Human Plasma

3.4 Summary of included studies

After analyzing 39 well-described studies that met our inclusion and exclusion criteria, two extraction techniques stood out in practical use during bioanalytical assays. In 52% of the included studies, the authors used LLE, while 41% used SPE. Only 7% had used less conventional methods to carry out their work and only 5% of the researchers used microextraction techniques (Figure 2).

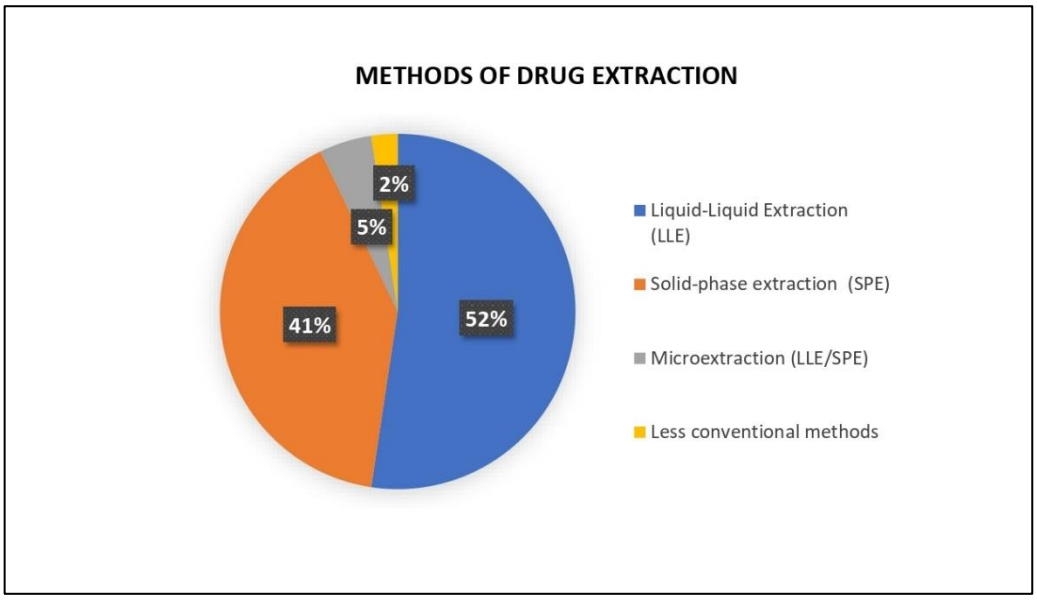


Figure 2. Synthesis of the results

3.5 Limitations of the scoping review process

Our scoping review has some limitations. To make this analysis more feasible, we included a random sample of analyzes performed on AINES, with diversified matrices. In this sense, our results can only be generalized to studies that focus on non-steroidal anti-inflammatory drugs. Furthermore, this scoping review was a huge undertaking and our results are only up to date until the year 2021.

4. Discussion

Sample preparation is one of the indispensable pillars of the science of analytical separation and LLE is among the simplest and most widely used sample preparation techniques. This fact was well observed in our review, as 52% of the studies present LLE as the technique of choice. The LLE is based on the transfer of solute from an aqueous

sample to a water-immiscible solvent, with extraction efficiency determined by the solute distribution coefficient between water and the receiving solvent (Tehranirokh *et al*., 2021).

According to Bitas et al., LLE is the technique most commonly used in sample preparation and the one that showed the highest selectivity among the simple solvent extraction methods (Bitas *et al*., 2018). It is probably the oldest of the techniques applied to determine various chemical compounds (Khatibi *et al*., 2022).

However, even though it has wide use and good analytical performance, LLE has several disadvantages such as emulsion formation, analyte loss, sample contamination, low sensitivity, automation difficulties, need for large sample volumes, and organic solvents (Bitas *et al*., 2018; Junza *et al*., 2014; Kechagia, Samanidou, 2017). At the present time, LLE is considered an expensive, time-consuming technique and does not meet the current requirements of green analytical chemistry (Alampanos *et al*., 2019).

Despite innovative trends in sample handling delve into the development of faster, safer, and more environmentally friendly extraction techniques, both LLE and SPE are still useful and widely accepted techniques for the exhaustive extraction of contaminants in organic or biological matrices (Quintana, Rodríguez, 2006).

This review also highlights that SPE is used as an analytical method in 41% of studies performed for LC-MS/MS assays. SPE is a classic and widely used extraction technique for biofluids and can be applied in manual, semi-automatic, or automatic format, such as the 96-well Hydrophilic-Lipophilic Balance SPE Block, widely used in bioanalytical assays. Through customized reports and the use of robotic systems, such as the Zymate XP robot, a storage carousel of SPE consumables and final extracts, which allows the construction of a bespoke SPE station, can be obtained (Alampanos *et al*., 2019).

In any case of application, the SPE compared to the LLE reduces the volumes of organic solvents used, in addition to the possibility of emulsion formation being strongly limited (Khatibi *et al*., 2021). However, the SPE demands an extensive and time-consuming procedure when compared to modern techniques such as SPME and Micro SPE, which would be a disadvantage, as the innovative techniques (but less used) eliminate the sample pre-treatment steps and the analysis time (Alampanos *et al*., 2019; Scott *et al*., 1999).

Nevertheless, Gjelstad, Rasmussen, Parmer and Pedersen-Bjergaard (2013) state that unfortunately the SPE is relatively expensive, the consumption of organic

solvents is considerable and the LC-MS/MS can still be subject to some interference from certain endogenous compounds (Gjelstad *et al*., 2013).

In this work, we also discussed that microextraction techniques have advantages over classical techniques (LLE or SPE), such as minimal use of solvents and reduced sample size, in addition to work optimization (Filippou *et al*., 2017; Toffoli, Lancas, 2015). In our analysis, we found two efficient microextraction techniques that are well employed in LC-MS/MS assays, namely, supramolecular magnetic solvent-based liquid-phase microextraction (SUPRAS) of hexafluroisopropanol (HFIP)-alkanol and online in tube solid-phase microextraction. These techniques represent 5% of our results.

Liquid phase microextraction based on supramolecular magnetic solvent (SUPRAS) of hexafluroisopropanol (HFIP)-alkane consists of immobilizing the extraction solvent. The hollow fiber has a cleaning ability, due to the microporous structure in the membrane wall. However, the filtering effect of hollow fiber is not sufficient to eliminate large molecules (e.g. proteins) in blood samples (Li *et al*., 2020).

The presence of large molecular substances in blood samples not only interferes with instrumental analysis, but also blocks the hollow fiber membrane pores, affecting the efficiency of mass transfer in the extraction process, and requiring a pre-treatment step. This step took place through SUPRAS, which is a type of water-immiscible nano/microstructural liquid originated from the self-assembly of amphiphilic molecular aggregates (micelles or vesicles), induced by specific environmental conditions (Ranjbar Banforuzi, Hadjmohammadi, 2017). In a simple and fast synthesis, the interactivity generated by SUPRAS can improve the extraction efficiency for a wide polarity of analytes. This excellent performance makes SUPRAS based on THF-alkyl carboxylic acid/alkanol widely applied in the pretreatment of various samples of complex matrices (Ballesteros-Gómez *et al*., 2009; Ranjbar Banforuzi, Hadjmohammadi, 2017). This new extraction method is simple, environmentally friendly and highly effective, and shows promising application potential in the analysis of blood samples and other complex samples (Li *et al*., 2020).

On the other hand, in-tube Solid Phase Microextraction, known in-tube SPME, is an effective sample preparation technique, as it makes use of an open tubular capillary column, as an SPME device, and can be coupled online with HPLC or LC-MS/MS (Kataoka *et al*., 2009).

It was developed to overcome problems related to the use of conventional SPME fiber, such as fragility, low sorption capacity and leakage. Unlike fiber SPME, tube SPME

typically uses a piece of fused silica capillary with a stationary phase coating on its inner surface (e.g., a small piece of column for gas chromatography) for extraction. The SPME in the tube is called “coated capillary microextraction”. This method directly extracts target analytes in aqueous matrices and concentrates the analytes in the stationary phase coated inside a capillary (Ahmadi *et al*., 2015; Kataoka *et al*., 2009; Toffoli, Lancas, 2015).

The analytes can be desorbed by introducing a mobile phase stream or using a static desorption solvent, with the analytes being more strongly adsorbed to the capillary coating. The desorbed compounds can later be injected into the LC column for analysis. The main advantage of this technique is the possibility of automating the SPME-HPLC process, allowing the extraction, desorption and injection to be carried out continuously, operating a standard automatic sampler that, when automated, reduces the total analysis time and is more accurate than the manual techniques (Ahmadi *et al*., 2015; Kataoka *et al*., 2009; Queiroz, Melo, 2014; Toffoli, Lancas, 2015).

The main disadvantage of the technique would be the need for very clean samples, as the capillary is easily clogged. Therefore, to avoid clogging the capillary column and flow lines, it is crucial to filter or centrifuge the sample solutions prior to extraction. Even though yields are usually low, such compounds must be extracted reproducibly, using an autosampler, and all extracts can be introduced into an LC column after SPME in the tube (Kataoka *et al*., 2009; Queiroz, Melo, 2014; Toffoli, Lancas, 2015).

In our search, we also see a totally new methodology, presented by (Gjelstad *et al*., 2013), for the preparation of biological samples, in particular for the miniaturized liquid-liquid extraction in a multiwell plate format, equivalent to 2% of our results (Gjelstad *et al*., 2013; Roldán-Pijuán *et al*., 2015).

In the PALME method, samples are loaded into individual wells in 96-well donor plates. Two 96-well plates, one donor plate and one receiving plate, where the analytes of choice are individually extracted through corresponding liquid membranes, each containing a few microliters of organic solvent and a volume of microliters of aqueous solution, are used to perform the extractions (Ask *et al*., 2018; Gjelstad *et al*., 2013; Roldán-Pijuán *et al*., 2015).

Introduced as an innovation in extraction techniques, this is an extension of LPME, which offers a simple workflow with high automation potential, being considered a contribution to “green chemistry, as it provides less use of organic solvent per sample, around 3–5 µL, working time of 15 to 30 minutes, in addition to proving to be a valid extraction method for basic hydrophobic drugs from human plasma, allowing

combinations with other methods and compatibility with LC-MS/MS (Ask *et al*., 2018; Gjelstad *et al*., 2013; Roldán-Pijuán *et al*., 2015).

PALME provided excellent sample cleanliness and is definitely susceptible to future automation and high throughput operation. Further development of PALME is expected soon, but for this to be successful, a fundamental understanding and more experimental data is needed. The authors hope to find a commercial supplier of 96-well plates with PALME-appropriate polypropylene membranes and definitively automate the process on an existing lab platform. It is also expected that it will be possible to run PALME on plates with more wells, for example 384-well plates (Ask *et al*., 2018; Gjelstad *et al*., 2013; Roldán-Pijuán *et al*., 2015).

5. Conclusion

This review presented and described several methods used by researchers to extract drugs from biological fluid samples, in particular NSAIDs, as an essential step for further analysis in LC-MS/MS.

The literature on the main methods used, LLE and SLE method, is extensive and consolidated, but we found other studies that report variations of these traditional techniques, equally validated for use in LC-MS/MS. From this review, it was concluded that the variation, reliability, and practical information regarding each analytical method used in this study can be adapted to advances in LC techniques, however, new approaches need to be explored in future research to address not only NSAID but also other drugs and matrices.

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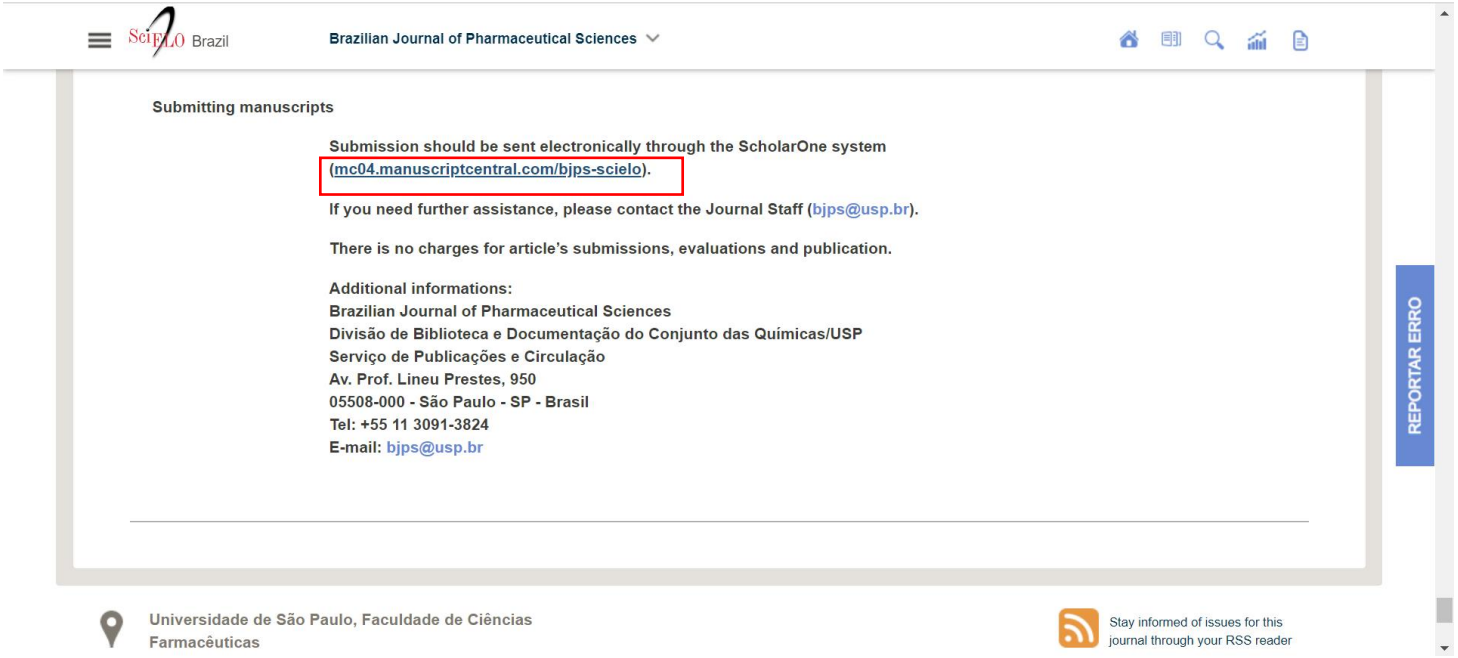
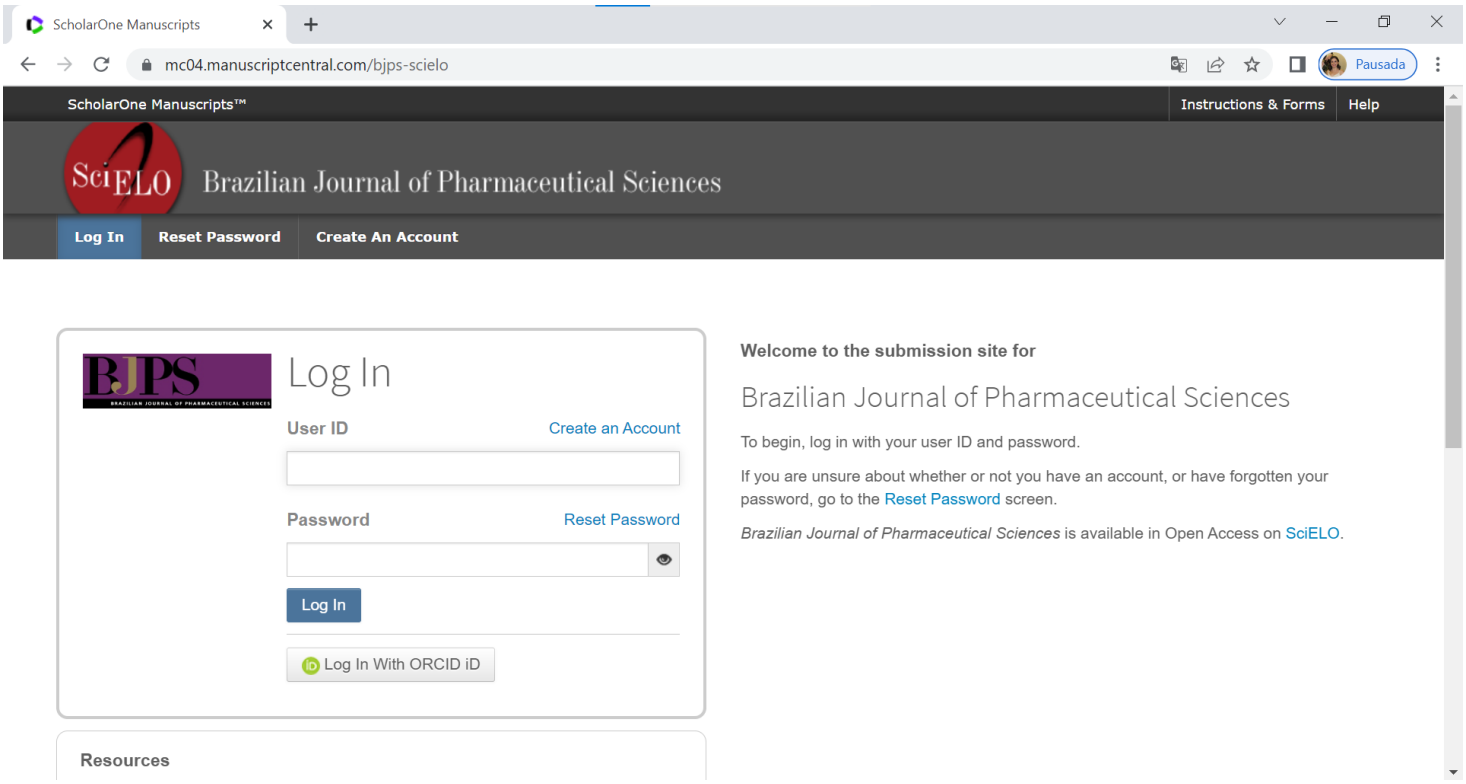
ANEXO

ANEXO A – Endereço de submissão



ANEXO B – Página de submissão



ANEXO C – Confirmação de submissão

Brazilian Journal of Pharmaceutical Sciences

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Submission Confirmation

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Analysis of different drug extraction methods in biological fluid samples for LC-MS/MS assays: Scoping review

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17-May-2022

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ANEXO D – Protocolo registrado e anexado no Open Science Framework

SCOPE REVIEW PROTOCOL FOR ANALYSIS OF DIFFERENT DRUG EXTRACTION METHODS IN BIOLOGICAL FLUID SAMPLES FOR LC-MS/MS ASSAYS

https://osf.io/nmjpy/

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Introduction

Mass Spectrometry (MS) is an analytical technique that identifies and quantifies unknown compounds or known materials with sensitivity at subnanomolar levels. In its beginning, during the 1950s, it was often used in conjunction with gas chromatography (GC), but its use was limited to the detection of small volatile molecules such as fatty acids, organic acids, amino acids, monosaccharides, prostaglandins, bile acids and steroids [1]. In recent years, there has been a growth in the MS area and in its use in clinical areas.

Liquid chromatography (LC) has been coupled with MS or MS/MS creating Liquid Chromatography in Mass Spectrometry (LC-MS/MS), which expanded its application to higher molecular weight or extremely polar molecules such as tetrodotoxin in human plasma and urine.

LC-MS/MS is a hyphenated technique, that is, it is a technique that allows a rapid determination of known substances with only a small amount of source material. The sample is first separated by High-performance liquid chromatography (HPLC) and

the effluent from the chromatography column is transferred to a mass spectrometer. LC separation is based on the physical and chemical properties of the analytes such as hydrophobicity, molecular size, presence of functional groups, etc. Therefore, when the effluent reaches the ion source in the mass spectrometer, ionization occurs and the mass spectrometer produces ions with signal intensity proportional to the amount of analyte present in the sample. In this hyphenated technique, the LC solves individual components of the sample in time, while the mass spectrometer selectively detects these components.

The manipulation or pre-treatment of the sample comprises one of the most time-consuming and laborious steps of the analytical procedures and for years it was considered of minor importance. The purpose of sample preparation is to remove matrix components that interfere with separation and/or detection and conversion of the analyte to a suitable form and concentration, increasing sensitivity. Today, with the improvements in chromatography and liquid chromatography columns, making the conversion of the analyte to a form suitable for separation is often unnecessary.

The peculiar nature of biological samples makes their preparation for LCMS/MS analysis a challenge in itself. Most clinical samples are aqueous (single matrices), but not all preparation techniques can be used for water-based samples, or belong to the group that typically includes multiple components that occupy or supply ions, such as hydrogen, sodium, and ammonium, causing ion suppression or enhancement in LC-MS/MS analysis. Salts and small hydrophilic molecules can have this effect, while compounds that affect droplet formation by acting as surfactant compounds in the matrix, and also affect ionization efficiency and result in ion suppression or enhancement. Finally, phospholipids, common in clinical specimens, are well known for their ion-suppressing effect. Therefore, the removal of these compounds is critical

for LC-MS/MS analysis. Examples of complex matrices are food, urine, plasma, and hair, among others.

In our study, we focused on the use of saliva as the analyzed biological matrix but also included other body fluids such as plasma, whole blood, and cerebrospinal fluid, among others. Saliva has gained attention as a new tool in clinical trials and monitoring of therapeutic drugs because it offers easy, noninvasive, stress-free, and real-time repeatable sampling, while blood or plasma collection is undesirable, as sometimes causes bruising, discomfort, or even are not performed by patients for fear, considering the multiple collections necessary. Therefore, analysis of analytes in saliva has great potential to make clinical research easier.

Objective

This study will be conducted to investigate and analyze different drug extraction methods in biological fluid samples for LC-MS/MS assays, following the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses for Scoping Reviews (PRISMA- ScR) Checklist.

Therefore, the following research question was formulated:

- What is the methodology and/or techniques most used for the extraction of non-steroidal anti-inflammatory drugs in bioanalytical assays using high-performance liquid chromatography and mass spectrometry (LC-MS/MS)?

Search for evidence

Data will be collected in PubMed, Lilacs, Embase, Scopus and Web of Science electronic databases. The End-Note reference manager will be used to save search

records and eliminate duplicate references. The selection of studies will be carried out by two reviewers independently so that, in the first step, titles and abstracts will be read and, in the second step, the full texts to filter only those that are actually compatible with the eligibility criteria. During all stages of study selection, a third reviewer was helping to resolve discrepancies.

Data extraction:

A table will be created for independent completion by two researchers. Information will be collected such as characteristics of the studies, country in which it was published, author's name, matrix used as biological fluid, equipment used, extraction technique used and which non-steroidal anti-inflammatory drugs used in each study.

ANEXO E – Página de acesso no Open Science Framework