Recent Advances in Understanding the Keys Factors Influencing Pressurized Liquid Extraction of Secondary Metabolites: A Comprehensive Review †

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Abstract: Pressurized liquid extraction (PLE) has become a pivotal technology for extracting secondary metabolites (e.g., phenolic compounds) from botanical sources, with implications spanning across pharmaceuticals, nutraceuticals, and functional foods. This communication provides an overview of recent advancements in understanding the key factors influencing the efficiency and selectivity of the PLE of these bioactive compounds. The optimization of PLE parameters, including pressure, temperature, and solvent characteristics, has been a focal point in recent research to enhance extraction yield and preserve the integrity of secondary metabolites. Investigations into the interaction of sample matrix properties, particle size, and solvent polarity have revealed nuanced effects on the extraction process, contributing valuable insights to developing targeted extraction protocols. Also, technological innovations, such as the utilization of green solvents and novel extraction techniques, are reshaping the landscape of PLE. Different studies exploring sustainable approaches not only enhance extraction efficiency but also align with the growing emphasis on environmentally friendly practices, paving the way for greener extraction processes. Collaborative efforts among researchers have led to a deeper understanding of the factors influencing PLE. Thus, this review highlights the recent progress in unraveling the complexities of the PLE of secondary metabolites, fostering a foundation for optimizing extraction methodologies and leveraging these bioactive compounds for diverse applications.

Keywords: pressurized liquid extraction; secondary metabolites; phenolic compounds; extraction parameters; optimization

1. Introduction

Secondary metabolites represent a diverse array of organic compounds synthesized by living organisms. While not directly involved in the primary metabolic functions necessary for growth and development, these metabolites play a pivotal role in the ecological and adaptive strategies of plants, microorganisms, and other organisms. Their significance extends beyond biology, as secondary metabolites have become indispensable in various industrial sectors. The extraction of secondary metabolites, with applications in pharmaceuticals, food, cosmetics, and agriculture, has seen significant advancements in recent years. Pressurized liquid extraction (PLE) emerges as a cutting-edge technique in this domain, offering a potent and efficient means of extracting bioactive compounds from various natural...
sources. This systematic review aims to comprehensively analyze and present the recent advances in understanding the key factors that influence the PLE of secondary metabolites intended for industrial applications. Thus, many studies highlight the pivotal role of PLE in enhancing extraction efficiency, providing a quicker and more selective method for obtaining pharmacologically active compounds [1,2]. According to Wijngaard and Brunton, this extraction technique has emerged as a promising technology to extract these bioactive compounds efficiently, ensuring the preservation of food products [3]. In addition, the findings of Alvarez-Rivera et al. in 2019 reveal that PLE, by its ability to maintain the integrity of sensitive compounds, has become an instrumental tool in their extraction for cosmetic applications [2]. Considered an environmentally friendly extraction method, PLE aligns seamlessly with sustainable agriculture principles [4,5]. In this systematic review, the synthesis of the recent literature will shed light on the nuanced factors influencing PLE from vegetal matrices in the recovery of secondary metabolites. Thus, the aim is to provide a comprehensive understanding of the current state of knowledge, identify research gaps, and offer insights into the future directions of this evolving field.

2. Methodology

This systematic review aims to compile recent advances in understanding the key factors influencing the PLE of secondary metabolites. The methodology follows a structured approach to ensure comprehensive coverage and critical analysis of the relevant literature. A search of electronic databases including PubMed, ScienceDirect, Web of Science, and Scopus was conducted to identify peer-reviewed articles published between January 2009 and May 2024. Keywords used in the search included “Pressurized Liquid Extraction”, “secondary metabolites”, “extraction parameters”, and “optimization”.

Relevant data from the selected studies were obtained using a standardized data extraction form. The data included study characteristics (author, year, source of secondary metabolites), PLE conditions (temperature, pressure, solvent, time), and outcomes (yield, biological activity of extracted metabolites). Extracted data were synthesized qualitatively and quantitatively. A meta-analysis was conducted to identify trends and correlations between PLE parameters and extraction efficiency to highlight the advances and gaps in our current knowledge.

3. Key Factors Influencing PLE

PLE, also known as accelerated solvent extraction (ASE), is a novel technique used in analytical chemistry to extract analytes from solid or semisolid samples. The principle of PLE lies in the synergistic effects of elevated pressure and temperature, which enhance the solubility of target analytes and facilitate their efficient transfer from the sample matrix to the liquid solvent [6,7]. As presented in Figure 1, the extraction efficiency of PLE is specifically linked to the matrix’s pre-treatment and characteristics, solvent selection, pressure, temperature, and instrumentation.

3.1. Sample Drying Methods

The choice of drying method plays a critical role in the effectiveness of PLE for obtaining bioactive compounds. Various drying techniques, including freeze drying, oven drying, and air drying, influence the physical and chemical attributes of plant materials, which in turn affects both the yield and stability of the extracted compounds. Freeze drying, which involves moisture removal at low temperatures and pressures, tends to better maintain the structure of heat-sensitive bioactive compounds compared to oven drying, which uses higher temperatures that can lead to compound degradation [8]. On the other hand, air drying, while more economical, may lead to oxidation and a decrease in volatile compound content [9]. Research indicates that different drying methods alter the matrix’s physical characteristics and porosity, thus influencing the solvent’s ability to penetrate and extract compounds during PLE [10]. Consequently, selecting the optimal drying
technique is crucial for enhancing the efficiency and effectiveness of PLE for bioactive compound extraction.

Figure 1. PLE critical factors influencing optimal recovery for industrial applications.

3.2. Matrix Pre-Treatment and Characteristics

Proper sample preparation is essential to homogenize the sample matrix and facilitate efficient extraction. Grinding, milling, or homogenization can break down sample particles, increase surface area, and improve solvent penetration. Removing interfering substances, such as lipids or proteins, through defatting or protein precipitation may also enhance extraction efficiency and reduce matrix effects [11,12]. The nature of the sample matrix can significantly affect the performance of PLE through its complexity, homogeneity, interactions, quantity, size, and moisture content.

So, understanding the influence of the sample matrix on PLE performance is essential for developing effective extraction methods, optimizing extraction conditions, and ensuring accurate and reproducible analytical results. Researchers often conduct systematic studies to evaluate the effects of different sample matrices on extraction efficiency and to develop strategies to mitigate matrix effects in PLE processes.

3.3. Solvent Selection

The choice of solvent plays a crucial role in the efficiency of secondary metabolite extraction. Different classes of secondary metabolites require specific solvent properties for efficient extraction. Polarity, selectivity, toxicity, strength, and compatibility with analytical techniques are the major characteristics of solvents impacting the extraction efficiency of secondary metabolite extraction.

Thus, the developments in novel solvents or solvent combinations for PLE equipment have focused on enhancing extraction efficiency, reducing environmental impact, and improving selectivity. Recent advances include (1) ionic liquids [13–15]; (2) deep eutectic solvents [16]; (3) natural deep eutectic solvents [17]; (4) solvent blends and co-solvents [18,19].

3.4. Pressure and Temperature

Pressure affects the solubility of analytes and the penetration of the solvent into the sample matrix. Higher pressures can enhance mass transfer rates and increase extraction efficiency. However, excessively high pressures may lead to sample degradation or matrix compression. The influence of temperature is illustrated by faster solvent penetration into the sample matrix and an increase in diffusion rate. However, the combined effect of pressure and temperature on PLE efficiency is complex and depends on the specific sample
matrix and analytes of interest. Optimization of both parameters is necessary to achieve maximum extraction efficiency.

3.5. Instrumentation

PLE instrumentation has seen several advancements aiming to improve extraction efficiency, throughput, and ease of use [12]. Some recent advancements include the following:

- Automation and integration;
- High-pressure capabilities;
- Temperature control;
- Pressure monitoring and regulation;
- Enhanced safety features;
- Compatibility with a variety of matrices;
- Reduced solvent consumption;
- Improved sample throughput;
- User-friendly interfaces and software;
- Miniaturization and portability.

Thus, recent advancements in pressurized liquid extraction instrumentation have focused on enhancing extraction efficiency, automation, safety, and user-friendliness to meet the evolving needs of analytical laboratories across various industries.

4. Optimal Extraction Parameters for Specific Secondary Metabolites from Different Plant Sources

As presented in Table 1, some authors have presented the optimal conditions to apply for specific secondary metabolites according to their sample matrices.

Table 1. Optimal conditions to apply for extraction of specific secondary metabolites.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Target Compounds</th>
<th>Key Extraction Factors</th>
<th>Yield</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Punica granatum peels</td>
<td>Phenolic acids, flavonoids, hydrolysable tannins</td>
<td>Ethanol/water 50:50 (v/v) (co-solvents)</td>
<td>200 10 20 Punicalagin: 22.0 ± 0.3 mg/g DW</td>
<td>[20]</td>
</tr>
<tr>
<td>Vitis vinifera L pomace</td>
<td>Monomers of flavans, flavonols, phenolic acids</td>
<td>Glycerol/water 15:85 (v/v) (co-solvents)</td>
<td>150 10 5 Catechin: 203.60 ± 12.22 µg/g DW</td>
<td>[21]</td>
</tr>
<tr>
<td>Betula pendula Roth leaves</td>
<td>Hyperoside, Betuloside</td>
<td>Ethanol 100% (polar solvent)</td>
<td>86 10 39 32% w/w; Hyperoside (54.1 mg/g), Betuloside (18.3 mg/g)</td>
<td>[22]</td>
</tr>
<tr>
<td>Myrciaria cauliflora peels</td>
<td>Anthocyanin</td>
<td>ChCl: Pro at 1:2 M ratio 47% (DES)</td>
<td>90 10 12 AR = 69.08 ± 2.62% w/w</td>
<td>[23]</td>
</tr>
<tr>
<td>Eucalyptus interecta leaves</td>
<td>Flavonoids, phenolic acids</td>
<td>Ethanol 26.6% (polar solvent)</td>
<td>179 36 52.3 g extract/100 g DW</td>
<td>[24]</td>
</tr>
<tr>
<td>Cannabis sativa L flowers</td>
<td>Terpenoids</td>
<td>Isopropanol (polar solvent)</td>
<td>75 10 5 B-pinene, Geraniol; --higher vs. conventional hand shakeout</td>
<td>[25]</td>
</tr>
</tbody>
</table>

Abbreviations: v/v: volume per volume; DW: dry weight; MPa: Mega-pascal; min: minutes; DES: deep eutectic solvent; AR: anthocyanin recover; ChCl: Pro: choline chloride and propylene glycol.

5. Conclusions

PLE remains a vibrant research area within analytical chemistry, continually evolving to meet the demands of various industries for efficient, selective, and environmentally friendly methods of recovering secondary metabolites. Novel advancements in the field of PLE are related to sample pre-treatments and characteristics, solvent selection and concentrations, pressure, temperature, and instrumentation.
Overall, these recent advances are characterized by combining fundamental research, technological innovation, and application-driven developments. As researchers continue to explore new methodologies, address challenges, and push the boundaries of PLE technology, the field holds significant promise for optimizing the extraction of biomolecules through green technology for further application in industries.


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**References**


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