Pressurized Solvent Extraction
Medicinal Plants and Natural Products

Pressurized Solvent Extraction (PSE) of medicinal plants and herbs for the determination of valuable compounds

Abstract

Pressurized Solvent Extraction (PSE) is a modern extraction method that has been developed as an alternative to traditional methods such as Soxhlet or maceration; it offers advantages with respect to extraction time and solvent consumption. BUCHI's SpeedExtractor enables the simultaneous extraction of six samples with maximum reproducibility and low solvent usage.

Polyphenols and active compounds were extracted from Edelweiss, St. John's Wort and Thyme. The results obtained with the SpeedExtractor are in accordance with the results obtained using reference methods or in the literature.

1. Introduction

Pressurized Solvent Extraction (PSE) is widely used in the research of medicinal plants and herbs. Typical applications are the quality control of active compounds in natural or pharmaceutical products and the research of valuable compounds in plant materials. Examples of the successful use of the SpeedExtractor E-914 / E-916 in academic research projects are: determination of rosmarinic acid in *Salvia* species [1], phenolic compounds and antioxidant activity in blackberry leaves [2], carrot metabolites falcarinol and falcarindiol in carrot leaves [3] and isoflavonoids in soy beans (*glycine* max.) [4].

Figure 1: *SpeedExtractor E-914 / E-916 combines maximum speed and throughput.*

The SpeedExtractor E-914 / E-916 is an automated instrument used for the parallel extraction of primarily organic compounds from a variety of solid or semi-solid samples. Conventional methodologies are accelerated using solvent at elevated temperatures. In order to maintain the solvent in a liquid state during the extraction process, the solvent inside the extraction cell is put under pressure. To achieve high recoveries multiple extraction cycles are usually applied. Once the extraction step is finished, the extracts are cooled down in a cooling unit and flushed into collection vials, which can then be easily evaporated in parallel using the Multivapor™ P-6 or Syncore Analyst R-12. The whole process workflow can be performed in parallel with up to six samples.

The instrument's methods offer a wide pressure and temperature range. The ability to make up solvent mixtures as required is particularly advantageous especially for the identification of actives in pharmaceutical products or for natural product extraction, as the extraction can be performed gently and quickly. As many plant compounds are sensitive to heat, relatively low extraction temperatures typically between 30-80°C are applied. The fact that the extraction is performed under elevated pressure enhances the extraction efficiency by destroying the matrix (cell structures) of the plant samples resulting in shorter extraction times and higher recoveries of the analytes.

Alternatively, the so-called "Pressurized (Hot) Water Extraction" or "Subcritical Water Extraction" has been successfully used to extract various, less heat-sensitive compounds from plant materials, e.g. from rosemary [5]. In this extraction method, water is used as the solvent at temperatures above its boiling point, e.g. 125-175 °C. The strong hydrogen bonds break at these subcritical temperatures as water becomes less polar and behaves more like an organic solvent such as methanol or ethanol.

There are many applications for the extraction of valuable compounds from plant materials for pharmaceutical or cosmetic purposes. Some of the studies carried out by BUCHI in collaboration with different universities are presented.

2. Determination of the total polyphenol content in Edelweiss [6]

Edelweiss (Leontopodium alpinum) grows in alpine areas, between 1800 and 3000 meters above sea level. Nowadays, it is also cultivated for its valuable extract. Edelweiss is known to be rich in polyphenols having a high antioxidising effect, which the plant has developed to protect itself against the intense UV-rays in its high alpine habitat. That is the reason why Edelweiss extracts are used in personal care and cosmetic products such as facial and sun creams [7].

Figure 2: *Edelweiss (Leontopodium alpinum) is cultivated for its valuable extract.*

The total polyphenol content is commonly used in plant analysis to quantify the power of the antioxidising effect. It is usually expressed as an equivalent of gallic

acid. The determination is performed photometrically according to Folin-Ciocalteu.

Table 1: *Determined total polyphenol content expressed as an equilvalent of gallic acid in Edelweiss (mean values, n=3)*

	SpeedExtractor	Literature [7]
Total Polyphenol Content	54.8 mg/g	50-60 mg/g

3. Determination of the total amount of hypericin in St. John's Wort (*Hypericum*) [8]

In herbal medicine St. John's Wort herb and capsules with dry extract are widely used for the treatment of depression. Hypericin one of the principal active constituents is determined for quality purposes.

The total amount of hypericin can be determined by the extraction and photometric quantification at 590 nm. The quantification of hypericin by photometry can be interfered by co-extracted chlorophyll. Removal of chlorophyll and extraction of hypericin can be done in a fast and reliable way with two consecutive extractions using pressurized solvent extraction on the SpeedExtractor E-916. Removal of chlorophyll was carried out by pre-extraction with DCM; the remaining residue was extracted with MeOH to quantify the hypericin.

Figure 3: *Colored extracts of pre-extraction (green) containing chlorophyll and of the main extraction (red) containing the hypericin*

Dried herb and capsules of a commercial drug product were analyzed. The results are presented in Table 2.

Table 2: *Determined hypericin content in St.John's Wort (mean values, n=3)*

	SpeedExtractor	Reference method [9]
Dried herb	0.8 mg/g	> 0.8 mg/g
Capsules	1.18 mg/ capsule	> 0.75 mg/ capsule

4. Simultaneous extraction of polyphenols and essential oils from thyme (*Thymus* ssp.) [10]

Thyme, *Thymus vulgaris* L., is part of the family Labiatae (Lamiaceae). Due to its various secondary plant constituents, thyme is used as a spice or as a medicinal plant. It was the medicinal plant of the year in 2006. The essential oils and the polyphenols that possess antioxidative properties can be extracted by pressurized liquid extraction (PLE) or pressurized water extraction (PWE). Both extraction techniques can be done with BUCHI's SpeedExtractor. The most important polyphenols in thyme are flavonoids and polyphenolic acids, such as luteolin, caffeic acid and rosmarinic acid [11].

Figure 4: *Thyme (thymus vulgaris)*

The goal of the study was to extract the valuable polyphenols (total polyphenolic content as well as the determination of rosmarinic acid) and the essential oils simultaneously using so-called pressurized water extraction, using water as a solvent at extraction temperatures above the boiling point. The use of water as a solvent is in accordance with the efforts to have "green laboratories" which includes the reduction of the consumption of organic solvent.

Pressurized water extraction using the SpeedExtractor E-914 is used to extract the valuable ingredients in thyme such as polyphenols and essential oils. It was shown that it is possible to extract simultaneously the water-insoluble essential oils and the water-soluble polyphenols using water as the extraction solvent under the conditions of the so-called pressurized water extraction. The combination of the extracted polyphenols with the essential oils gave a pleasant overall taste impression, which had the highest impact, with a typical fresh and cooling thyme note when extracted at 125 °C. In order to prevent the degradation of thermolabile substances and to avoid the formation of probable Maillard-type reaction products, that have negative impact on the taste of the extract, the temperature should not be set above 125 °C during the extraction.

Table 3: *Simultaneous determination of polyphenols and essential oils in dried thyme. Determined contents of total polyphenol content (expressed as an equivalent of gallic acid), rosmarinic acid and essential oils with extraction parameters of 125°C, 150 bar, (mean values n=3, for rosmarinic acid n=4)*

	Determined contents
Total Polyphenol content (mg/g)	45.6
Rosmarinic acid (mg/g)	4.9
Essential oils (v/w %)	1.2

In order to investigate the influence of the extraction temperature on one of the main polyphenols in thyme (rosmarinic acid), HPTLC analysis was performed. The influence of different extraction temperatures on the content of the rosmarinic acid is shown in Figure 5. At an extraction temperature of 125 °C, the extraction yield was highest at 4.90 mg rosmarinic acid/g thyme. Further increase in the extraction temperature led to a decrease in the concentration of the rosmarinic acid, which is most probably due to the degradation of the acid.

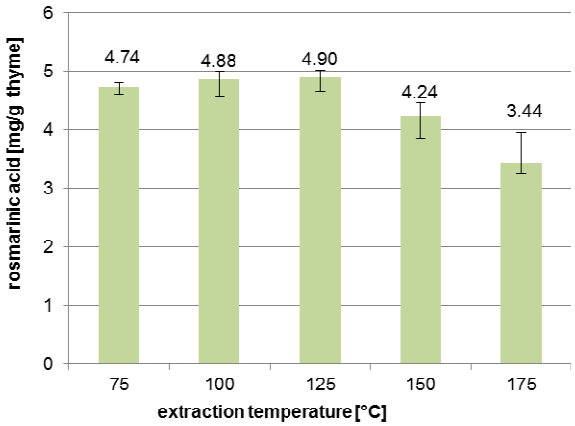

Figure 5: *Determination of rosmarinic acid at different extraction temperatures using PWE. The highest yield was obtained at an extraction temperature of 125 °C.*

5. Conclusion

BUCHI's SpeedExtractor E-916/E-914 is ideal for the pressurized solvent extraction of medicinal plants and herbs. With its parallel format, up to six samples can be extracted simultaneously, giving a high sample throughput with maximum reproducibility. The applied pressure allows the plant cells to be broken up and the analytes more easily released, leading to faster extractions with low solvent consumption.

Polyphenols and other active compounds were extracted from different plant materials, namely from Edelweiss, St. John's Wort and Thyme. The results obtained with the SpeedExtractor E-916/E-914 are in accordance with the results obtained using reference methods or in the literature.

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7. References

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8. Additional information

- BUCHI Application notes 003/ 2009 and 004/2009 Extraction of Genepi (*Artemisia umbelliformis*) for the Determination of Costunolide and Total polyphenol content
- Case study "Extraction of potential drugs", University of Pharmacology, France
- Case study "Separation of plant extracts to isolate saponins", EcoSafe Natural Products Inc., Canada
- Case study "Sepacore® for the separation of plant extracts", University of Basle, Switzerland.
- Case study "Purification of Synthetic & Natural Compounds", Central Drug Research Institute, India

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