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Journal of Chromatography A, 976 (2002) 345–348

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Automated sample preparation by pressurized liquid extraction–solid-phase extraction for the liquid chromatographic–mass spectrometric investigation of polyphenols in the brewing process

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Abstract

The analysis of polyphenols from solid plant or food samples usually requires laborious sample preparation. The liquid extraction of these compounds from the sample is compromised by apolar matrix interferences, an excess of which has to be eliminated prior to subsequent purification and separation. Applying pressurized liquid extraction to the extraction of polyphenols from hops, the use of different solvents sequentially can partly overcome these problems. Initial extraction with pentane eliminates hydrophobic compounds like hop resins and oils and enables the straightforward automated on-line solid-phase extraction as part of an optimized LC–MS analysis.

Keywords: Food analysis; Polyphenols

1. Introduction

Polyphenols, a large group of secondary plant metabolites, are of importance to the brewing process as natural ingredients of hops and malt [1–3]. Their ability to interact with proteins forming cross-linked aggregates has a strong influence on haze formation. Their antioxidant properties may prevent staling and account partly for the aroma stability of beer. Due to their antioxidative capacity, their health potential has been discussed in recent years. Yet only little information is available focusing on individual compounds, their possible modifications in the brewing process and their specific contribution to protein interactions. Specific analysis of individual phenolic compounds is complex due to their low concentrations in sample material and a complex matrix. Conventional procedures like solid–liquid and liquid–liquid extraction account for analyte loss and deliver extracts with usually a high background of interfering hop resins and oils.

HPLC–MS–MS proved to be a valuable tool for identification of phenolic compounds [4], and sample preparation is of due importance to increase sensitivity and reduce matrix effects. Accounting for the high activity and their antioxidant properties, isolation of phenolics from sample material and from the majority of matrix interferences has to be rapid and careful. Pressurized liquid extraction (PLE) increases efficiency with a reduction of time and solvent using high pressure and elevated temperature for the extraction of solid sample materials. Solid-phase extraction (SPE) has a high efficiency in selectively concentrating and purifying analytes from crude extracts in respect to undesired interfering compounds. On-line coupling of PLE, SPE and HPLC

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0021-9673/02/$ – see front matter
PII: S0021-9673(02)01230-X

offers automated and highly reproducible analysis with a minimum of manual work required. In addition to a previous development of automated sample preparation for malt [5], we applied the on-line coupling of PLE and SPE to the analysis of polyphenols from hops.

2. Materials and methods

The new technique of on-line coupling of PLE, SPE and HPLC has been presented for the analysis of malt samples in detail previously [5] and some details are shown in Fig. 2. Pressurized liquid extractions were carried out on an Automated Sample Extractor ASE200 (Dionex, Idstein, Germany) using 11-ml stainless steel extraction cells. Extracts were purified by Automated Sample Preparation with Extraction Cartridges (ASPEC; Abimed, Langenfeld, Germany) using commercially available polyamide cartridges (1 g/6 ml, Macherey–Nagel, Düren, Germany). Prepared samples were then subjected to high-performance liquid chromatography with UV and MS–MS detection, using a System Gold chromatograph (Beckman Coulter, Unterschleißheim, Germany) and an LCQ ion-trap mass-spectrometer (ThermoFinnigan, Egelsbach, Germany). Solid sample materials analyzed were hop pellets (provided by NATECO$_2$, Wolnzach, Germany).

Hop pellets were ground in a mortar and 1 g used for each extraction, mixed with 2 g diatomaceous earth (Isolute HM-N, Separtis, Grenzach-Wyhlen, Germany) and the remaining free space of the cell was filled with diatomaceous earth. PLE extraction at 60 °C and 10 min using acetone–water (4:1, v/v) as solvent was carried out with and without pentane preextraction (60 °C, 10 min, two cycles). For SPE, PLE extracts with pentane preextraction were diluted with water prior to SPE to reduce the acetone content, the cartridge washed with water after application of the sample and dimethyl formamide (DMF)–water (85:15, v/v) used for elution. For extracts without pentane preextraction, interferences were either removed by liquid–liquid extraction with chloroform or by centrifugation of the precipitate after the addition of water as described above prior to SPE.

3. Results and discussion

Temperature, extraction time and solvent are of the greatest influence for PLE efficiency. Using different solvents sequentially can improve purification by selectively removing interferences in the first step, prior to extraction of desired analytes. For optimization of the whole analytical process, however, compatibility with subsequent SPE has to be taken into account.

In accordance to the results for extraction of phenols from malt [5], a temperature of 60 °C and a time of 10 min was optimal, using acetone–water (4:1, v/v) as solvent. However, the high acetone content of this solvent is incompatible with SPE purification, and without further measures, dilution of the extract with water precipitates a large amount of lipophilic compounds, presenting the major obstacle in a straightforward procedure. These precipitates might enclose analytes of interest and render it impossible to transfer the extracts onto the SPE cartridge. On the other hand, extraction efficiency was found to decrease rapidly with lower acetone percentage (results not shown).

Conventionally, interferences are removed after acetone–water extraction through liquid–liquid extraction with chloroform, evaporation of the acetone with subsequent filtration and polyamide SPE. Alternatively, extraction is carried out with water as extraction solvent, preventing precipitation problems but extracting only part of the polyphenols present in the sample.

The use of different solvents sequentially can overcome these difficulties and is of great advantage for the isolation of the analytes from matrix interferences. Hops as well as hop pellets and other hop preparations present a high content of chlorophylls, hop oils and resins, interfering with analytes as well as accounting for these problems in subsequent solid-phase extraction. In ASE, drying the sample with nitrogen after extraction makes it possible to use two immiscible solvents in sequence, which is not straightforward with manual extraction. Matrix interferences can be largely reduced with an initial pentane extraction (60 °C, 10 min, two cycles), which will not extract phenolic analytes along with the matrix. Fig. 1 gives a comparison of the extraction of phenolic constituents by PLE with and

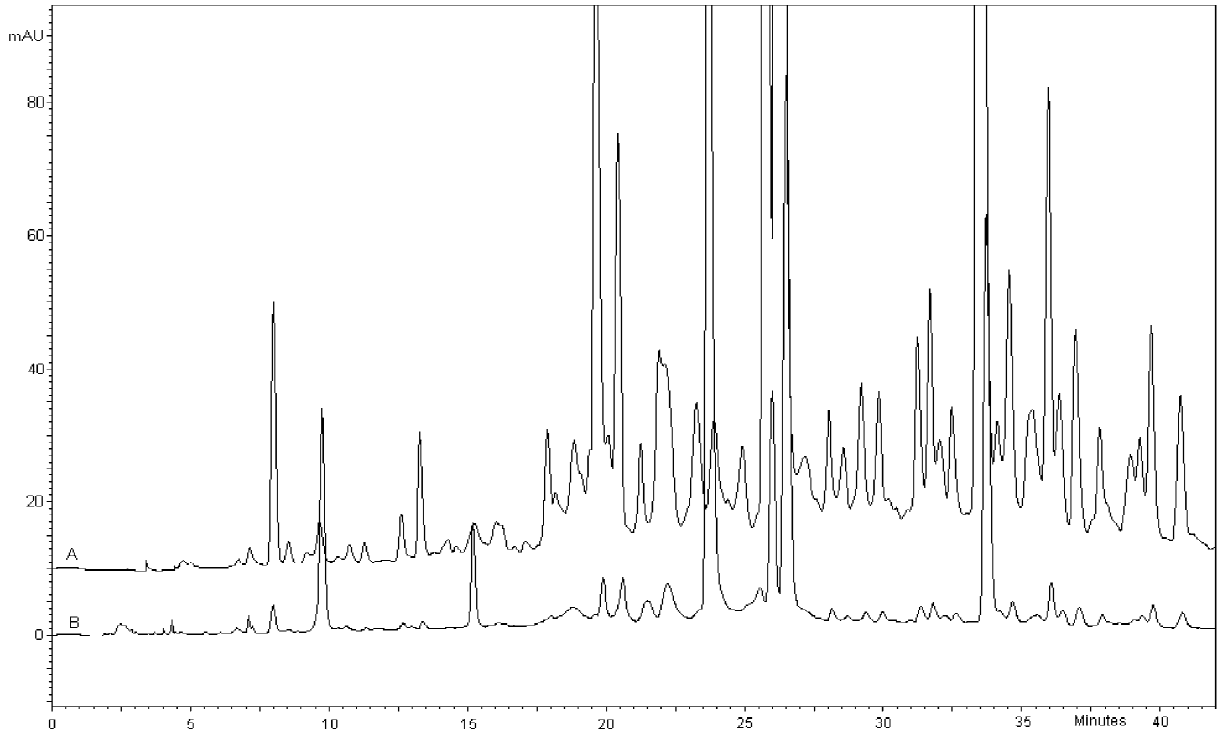

Fig. 1. HPLC chromatograms (UV detection at 280 nm) demonstrating pentane preextraction by PLE. Samples of hop pellets were extracted by PLE and purified by SPE. Interfering compounds in A (without pentane preextraction) were removed in B (with pentane preextraction). The key compounds (individual peaks not marked) are hydroxybencoic and hydroxycinnamic acids (5–20 min) and quercetin and kaempferol glycosides (20–35 min).

without pentane preextraction with a markedly reduced matrix background in the latter.

In addition to PLE of phenolic compounds from hops, SPE purification of extracts with polyamide cartridges was optimized in respect to solvents and volumes used for washing and elution. Investigations are still underway to enhance the retention of desired compounds on the cartridge. However, it is already obvious that the use of commercial SPE cartridges in contrast to hand-packed columns as previously used and the optimization of SPE parameters offers great potential.

The online coupling of PLE and SPE offers streamlined extraction and purification without manual work required in the process. The ASE and the ASPEC robot have both been modified, so that the ASPEC can automatically take over extracts from the PLE system. Before withdrawal, the ASPEC dilutes the extract with water to a solvent content compatible with adsorption to the SPE material. This is only feasible using pentane preextraction, thus preventing precipitation of hop chlorophylls, resins and oils. After SPE purification and fractionation, the ASPEC injects the SPE extract into the HPLC system. This new instrumental set-up has been described in detail in Ref. [5]. Fig. 2 compares the former manual with the new automated method. Beside the benefits in efficiency and selectivity of the extraction, the automated method saves time and manual work and enhances reproducibility.

4. Conclusion

The relatively new PLE technique can be used efficiently for the analysis of phenolic compounds from hops. Extraction efficiency is higher compared to manual extraction, with a largely decreased

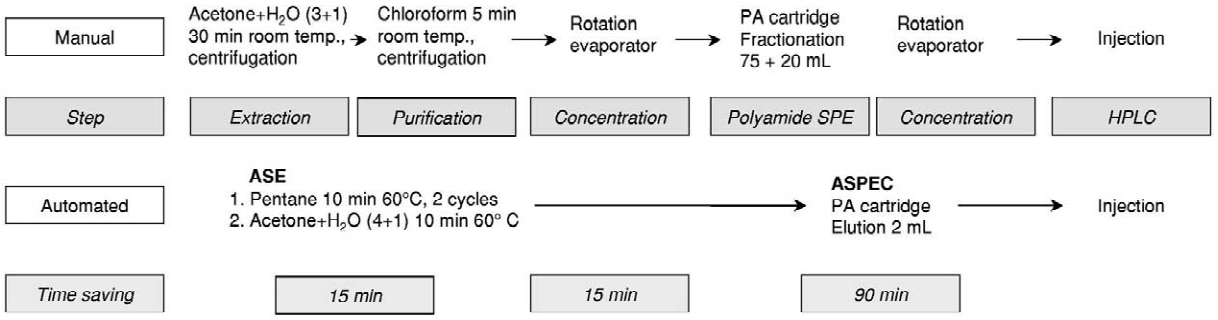

Fig. 2. Comparison of the manual and the automated sample preparation of hop pellets indicating the time saved by automation.

amount of matrix interferences. Pressurized liquid extraction delivers highly concentrated extracts, is much faster than manual extraction and reduces subsequent time-consuming steps like solvent evaporation, thus minimizing the possibility of alteration and degradation of sample compounds.

Further investigations will focus on additional optimization of PLE and purification by SPE to further enhance selectivity for phenolic compounds in the sample preparation process. The method will help to investigate the fate of different polyphenols in the brewing process and their role in haze formation.

Acknowledgements

We thank the staff at Dionex and Abimed, namely Dr. G. Maio and Dr. M. Krappe, who developed the ASE–ASPEC-coupling, and Dr. C. Behn for their active support. We also appreciate the cooperation with NATECO$_2$ hop extraction, Wolnzach, Germany. The financial support by the AiF (project AIF 12605) and WiFö (Wissenschaftsförderung der Deutschen Brauwirtschaft e.V.) is kindly acknowledged.

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