APPLICATION OF ULTRASOUND-ASSISTED SOLVENT EXTRACTION OF POROUS MEMBRANE PACKED LIQUID SAMPLES FOR POLYPHENOLS DETERMINATION IN WINE SAMPLES.

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Abstract

Polyphenols play a crucial role in a proper human health maintenance as well as their presence very often correspond to the quality assessment of producs like wine. Thus, their monitoring is of high interest. However, as they occur in a complex matrices their extraction is very often necessary prior the analysis. Herein, a new ultrasound-assisted solvent extraction of porous membrane packed liquid sample technique has been optimized for the determination of polyphenols in wine samples as an alternative for existing methods used prior gas chromatography – mass spectrometry analysis.

Achieved accuracy is in the range of 100.7 – 108.3 while recovery between 97 – 110% from spiked samples at 5 to 10 ppm concentration range. LOD ranges between 0.174-1.99 µg/mL while LOQ 0.522-5.97 µg/mL.

Key words: wine, polyphenols, extraction,

1. Introduction

Increased awareness of the society about the positive influence of the application of a healthy and well balanced diet is a driving force for scientists looking for bioactive compounds promoting human health as well as factors influencing their occurrence and availability. Natural antioxidants are the subject of high interest during the last decade. The most widely studied group of compounds having desired properties are polyphenols. Apart from antioxidant properties they exhibit other health beneficial

effects like anti-inflammatory, antimutagenic, anticarcinogenic as well as they help to prevent against age-related diseases [1]. It is well known, that polyphenols are widely abundant in fruits especially blueberries, grapes and products made from them. Thus, wine is considered as one of the source of given compounds, where they play a crucial role, being responsible for the aesthetic and organoleptic character of this alcoholic beverages. Thus, it is important to monitor the content of polyphenols to control the quality of wine products. There are plenty of methods of polyphenols determination described in literature. However, mostly they are based on high performance liquid chromatography (HPLC) and capillary electrophoresis. Gas chromatography (GC) was applied only in few cases. This is due to the physico-chemical properties of given compounds and thus more complex sample preparation process than in HPLC, since extraction of preferable analytes and their derivatization should be performed[2]. Herein, in a given study a new, promising extraction technique is presented an ultrasound-assisted solvent extraction of porous membrane packed liquid sample. Following work is focused on catechin, rersveratrol and pterostilbene determination in the red wines originated in Poland.

2. Experimental

2.1 Reagents and chemicals

All standards were of high purity, suitable for the GC analysis, (+)-Catechin were purchased from Sigma-Aldrich. Resveratrol and Pterostilbene were delivered from Extrashynthese (France). Additionally, the derivatization reagent 1% trimethyl chlorsilane (TMCS) and N,Obis(trimethylsilyl)trifluoroacetamide (BSTFA) were supplied by Sigma Aldrich. Polypropylene (PP) flat membrane sheet (Type PP 1E (R/P), pore size: 0.1 μm, wall thickness: 100 μm) was purchased from Membrana (Germany). Deionized water from the Milli-Q Direct 8 Water Purification System (Merc Millipore) for the sample (pre)treatment and sample dilution was used.

2.2 Samples

10 bottles of red wines originated from Polish vineyards.

2.3 Instrumentation

Analysis was performed with 7890A GC System (gas chromatography; Agilent Technologies, Santa Clara, CA, USA) coupled to an electron ionization (EI) ion source and a 5975C single quadrupole mass

spectrometer (MS) (Agilent Technologies), by which resveratrol, pterostilbene and catechin concentration were investigated.

2.3 Extraction and derivatization procedure

New, alternative extraction technique procedure is presented on Fig. 1. Firstly, the membrane bag with the sample needs to be prepared. Secondly, porous membrane bag is subjected to the solvent extraction assisted by the ultrasounds bath for 25 min and thirdly, it is evaporated in the stream of nitrogen. Afterwards, the derivatization process should be performed in order to enhance the volatility, thermal stability of analytes as well as to improve resolution and detection of the gas chromatography performance. During the derivatization process 30 µL of BSTFA was added in the vial and vortex for 30 s. Than the solution was heated for 30 min in the temperature of 35˚C. At the end 170 µL of extraction solvent was added and heated again at the same condition for 15 min prior analysis.

3. Results and discussion

Matrices of wine samples are very divers apart of polyphenols contains sugars, tannins, vitamins, organic acids, minerals, aromatic compounds and many others that disturb the analysis of a selected compounds. Due to this fact sample preparation is a very crucial aspect. Up till now, there were several extraction techniques applied, like: Solid Phase Extraction (SPE), Solid Phase Microextraction (SPME), Stir Bar Sorptive Extraction (SBSE) or Dispersive Liquid-Liquid Microextraction (DLLME) [3-7]. However, there is no unique technique that can be applied to each kind of matrix so still there is a place for new one.

Presented in this paper, new extraction technique is easy in sample and extraction device preparation and consumes minimal amount of solvent. There were several parameters optimized like the type of extraction solvent, its volume (1 ml of EtAc:DCM (1:1)), time required for sufficient extraction and ultrasounds power. After optimization of a given parameters, developed extraction technique gives significant improvements over the published method, having a promising results expressed in the validation parameters as well as precision and recovery presented in Table 1 and Table 2. LOD is in the range between 0,174-0,329 µg/mL while LOQ is within 0,522 – 5,97 µg/mL. Moreover, accuracy is in

the range of 102.2 – 114.7 while recovery between 95,8 – 110%. Those are very good analytical figures of merit, showing reliable and reproducible results in terms of polyphenols determination.

Additionally, there is no need of special solvent to be used. Extraction and desorption is possible within one single step. There is no need of salt addition and pre-condition treatment to μ-SPE device what in total make this technique economically favorable. As every extraction technique also this one has some limitations since once used the membrane bag cannot be used again. Special care needs to be taken while processing, because the μ-SPE device can break during the extraction process. Up-till now, automated procedures for device fabrication, extraction and its online application with analytical instruments are not available yet. However, it is possible to couple ultrasound-assisted solvent extraction of porous membrane packed liquid sample technique with different quantification techniques.

4. Conclusions

Wine is a complex matrix rich in many different groups of compounds thus extraction of desired analytes is a crucial aspects in the context of wine sample analysis. There are several extraction techniques already available nevertheless there is no one uniqueness, ideal for each kind of analytes extraction from a sample thus the door is still open for new techniques which may eliminate limitations previously appearing. Presented extraction techniques of polyphenols from wine matrices is a promising tool for the complex matrix sample preparation prior the gas chromatography analysis. Validation process of a given technique clearly shows that method can be successfully used for the quantitative measurements of polyphenols in wine samples, giving reliable and reproducible results.

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Figures

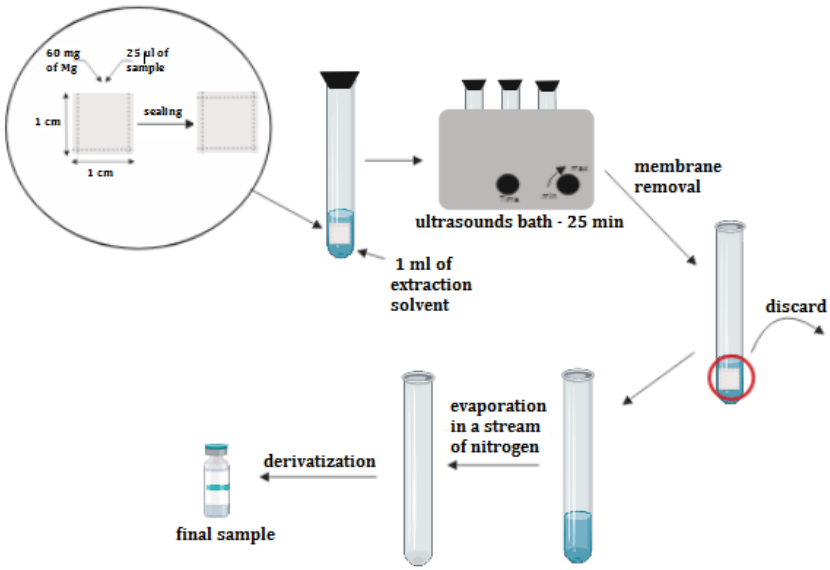

Figure 1Scheme of the ultrasound-assisted solvent extraction of porous membrane packed liquid samples extraction process.

Tables

Table 1 Method validation parameters

Compounds	Linear range (µg/mL)	r^2	r	LOD (µg/mL)	LOQ (µg/mL)
Pterostilbene	0.58 - 29	0.9979	0.9989	0.174	0.522
Resveratrol	0.987 - 24	0.9966	0.9983	0.329	0.987
Catechin	5.97 – 14.18	0.9884	0.9942	1.99	5.97

Table 2 Method precision and recovery

Compounds	**Intra-day precision Accuracy (precision) (n = 7) (c = 10 µg/mL)**	**Recovery %R ± $U_{\%R}$(k =2) (n = 5)**		**ME Accuracy (precision) (n =3) (c = 5 µg/mL)**
				Red wines
Pterostilbene	100.7 (9.0)	5 ppm	102.3 ± 2.9	102.2 (5.0)
		10 ppm	110.0 ± 11.7	
Resveratrol	105.0 (7.5)	5 ppm	99.2 ± 16.6	114.7 (7.9)
		10 ppm	106.0 ± 13.1	
Catechin	108.3 (6.8)	5 ppm	95.8 ± 11.2	106.5 (2.3)
		10 ppm	97.2 ± 12.3	