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Utilization of coulometric array detection in analysis of beverages and plant extracts

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Abstract

This paper summarises the findings obtained by using multi-electrode detector CoulArray in HPLC analysis of biologically active compounds. The methods for analysis of selected phenolic compounds were optimized. Optimal conditions of separation and detection were applied to real sample analysis. White and red wines as well as meads were analyzed and the content of phenolic acid was evaluated. Further the roots of Japanese knotweed were analyzed.

Keywords: CoulArray detector; phenolic acids; stilbenes; wine; mead; Japanese knotweed.

1. Introduction

High performance liquid chromatography (HPLC) is analytical technique widely used in analysis of natural compounds including antioxidants. HPLC with different types of detection is possible to use in dependence on substances properties. Common types of detectors used in HPLC are spectrophotometric, fluorescence, electrochemical and mass. HPLC equipped with electrochemical detection has become popular due to its high selectivity as well as sensitivity [1-4]. CoulArray detector is coulometric multi-electrode electrochemical detector for HPLC. The instrument was designed to detect and quantitate trace levels of electroactive compounds in complex matrices. The CoulArray detector uses a multi-electrode detector system in which a series of electrochemical cells (4 – 16) are set at different potentials to oxidize (or reduce) the compounds that elute from the column. In Fig. 1 is depicted a scheme of electrochemical transformation of substances A and Y at different potentials which are set to electrochemical cells. This approach allows for the collection of a number of chromatograms (one chromatogram is generated at each potential) rather than a single chromatogram, and thus allows for the identification of the compounds of interest based on the retention time and its oxidation (reduction) characteristics [5,6]. For each compound, its specific hydrodynamic voltammogram is obtained and used for the peak identification.

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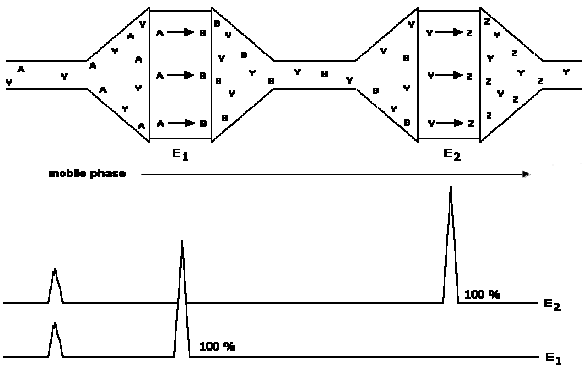

Fig. 1. Scheme of electrochemical transformation of substances A and Y in electrochemical cell.

Biologically active compounds are the subject of interest of the scientists and also of the public in last several years [7-11]. It is well known, that these compounds with antioxidant (and many others) effects occurring in nature are secondary metabolites of plants, which are synthesized as a defence response to situation of stress, such might be microbial infection, UV irradiation or mechanical damage.

Among biologically active compounds widely studied belong e.g. phenolic acids – derivatives of benzoic and cinnamic acid, catechins or stilbenes [12-15]. Beneficial effects on human health were evidenced in these groups of substances. In Fig. 2 are depicted the structures of these compounds.

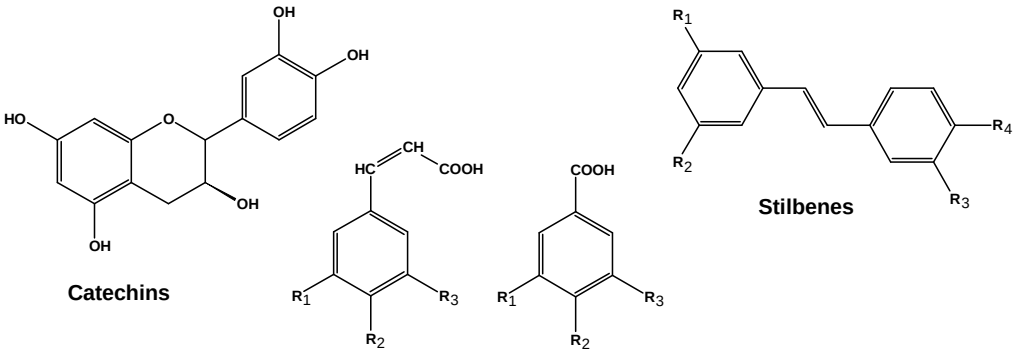

Fig. 2. Structures of analyzed compounds.

The compounds from these groups of naturally occurring antioxidants were analyzed in this work in the samples of wine, meads and also plant Japanese knotweed using technique HPLC equipped with electrochemical CoulArray detection.

2. Experimental

2.1. Apparatus

A HPLC system (ESA Inc., Chelmsford, MA, USA) was composed of two gradient pumps, thermostatic chamber and coulometric detector CoulArray, which consisted of two cell packs in series, each pack containing four porous graphite working electrodes with associated palladium reference electrodes and platinum counter electrodes. For sample preparation an ultrasonic bath Sonorex RK 31 (Bandelin Electronic, Berlin, Germany) was used.

2.2. Chemicals

Standards of phenolic acids such as gallic, 4-hydroxybenzoic, protocatechuic, 4-hydroxyphenylacetic (4-HPAC), vanillic, chlorogenic, sinapic and coumaric were purchased from Fluka (Buchs, Switzerland). Gentisic acid, syringic acid, caffeic acid and ferulic acid were obtained from Sigma Aldrich (Prague, Czech Republic). Catechin, epicatechin, protocatechualdehyde, vanillin, ethylvanillin and rutin were purchased from Fluka (Buchs, Switzerland). Standard compound from group of stilbenes were purchased from various suppliers. Resveratrol was purchased from Sigma Aldrich (Prague, Czech Republic), astringin and piceid were purchased from Sequoia Research Products (Pangbourne, UK) and piceatannol was purchased from MoBiTec (Göettingen, Germany).

Other used chemicals were as follows: ammonium acetate (Fluka, Buchs, Switzerland), formic acid (Lachema, Brno, Czech Republic), acetonitrile (gradient grade) and methanol (both from Merck, Darmstadt, Germany).

2.3. Samples

The samples of red and white wines were obtained from Moravian wine growers. Meads were obtained from Czech beekeepers. The roots of plant Japanese knotweed were obtained from Botanical Institute of Academy of Sciences, Czech Republic.

2.4. Sample preparation

The samples of wine were placed into ultrasonic bath for 15 minutes, then diluted with mobile phase (1:4), filtrated through 0.22 μm filter and analyzed. The samples of meads were diluted (1:5 – 1:30, in dependence on type of mead) and filtered through 0.45 μm membrane filter and analyzed. The extracts of plant Japanese knotweed were prepared from the dried powder of the roots. This powder was mixed with suitable solvent (50 % methanol) and placed into ultrasonic bath for period 15 minutes. The extracts prepared were filtered through 0.45 μm membrane filter and analyzed.

2.5. Chromatographic analysis

The conditions of chromatographic analysis were very similar for all analyzed samples. Chromatographic separations were performed on LiChrospher 100, RP-18 (125×4 mm i.d., 5 μm particle size) column and Gemini C18 110A (150×3 mm i.d., 3 μm particle size) column (both from Merck, Darmstadt, Germany). The mobile phase consisted of two component parts: (A) and (B). Mobile phase (A) consisted of 5 mM ammonium acetate in water acidified by adding formic acid. Mobile phase (B) was acetonitrile or its mixture with water and 5 mM ammonium acetate, respectively. The flow rate was 0.4 mL min^{-1} in gradient elution. The individual gradient elution was optimized for samples of wine, meads and Japanese knotweed. The temperature of separation and detection was 35 °C. Potentials of eight electrochemical cells for detection of compounds were set in the range 200-900 mV with 100 mV step.

3. Results and discussion

3.1. Representative chromatograms

Within the framework of this study about the utilization of coulometric array detection 30 samples of Moravian wines were analyzed. Two sorts of wine – white and red – were examined for a content of selected phenolic compounds. Further 22 samples of meads were investigated. Possibility of use coulometric array detection in plant analysis was examined on extracts prepared from roots of Japanese knotweed. Representative chromatograms are depicted in Figs. 3-6. Individual chromatograms (8) in each picture present a records at potentials monitored.

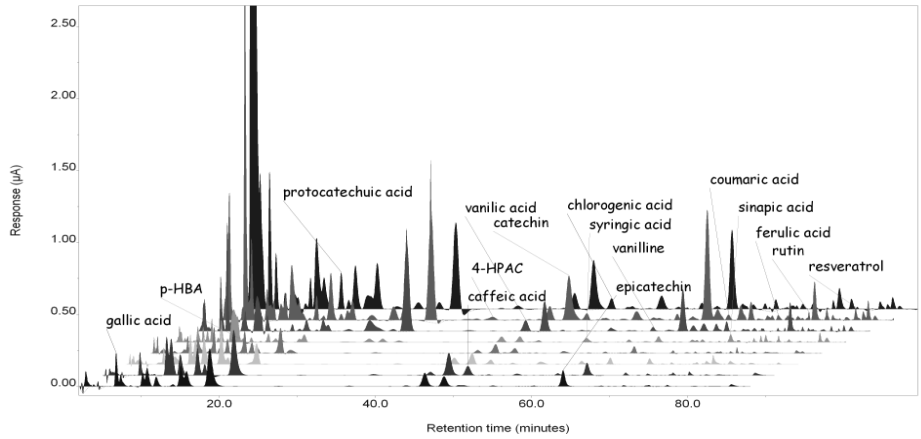

Fig. 3. Chromatogram of white wine (Rulandské bilé, 2003), Condition: Column – Lichrospher® 100, RP-18 (125 x 4 mm, 5μm), mobile phase – aqueous 5 mM ammonium acetate with acetonitrile under gradient program, flow rate 0.4 mL/min, column and cells temperature 36 °C, detection 250, 300, 400, 500, 600, 700, 800 and 900 mV.

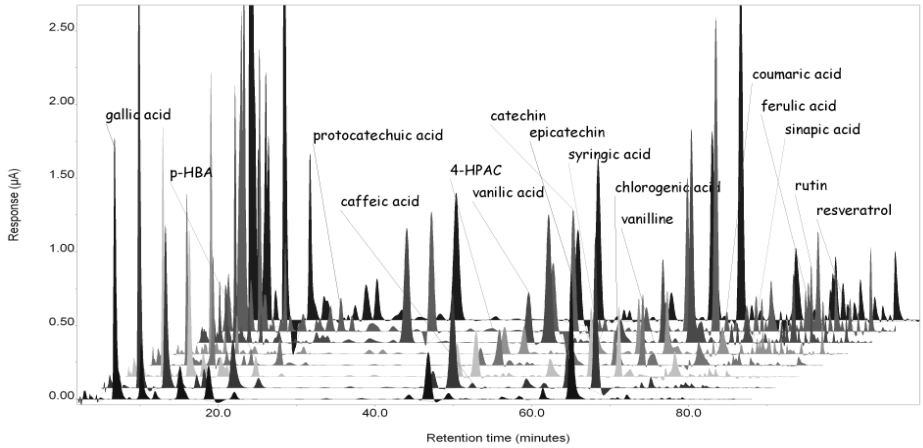

Fig. 4. Chromatogram of red wine (Modrý Portugal, 2002), Condition: Column – Lichrospher® 100, RP-18 (125 x 4 mm, 5μm), mobile phase – aqueous 5 mM ammonium acetate with acetonitrile under gradient program, flow rate 0.4 mL/min, column and cells temperature 36 °C, detection 250, 300, 400, 500, 600, 700, 800 and 900 mV.

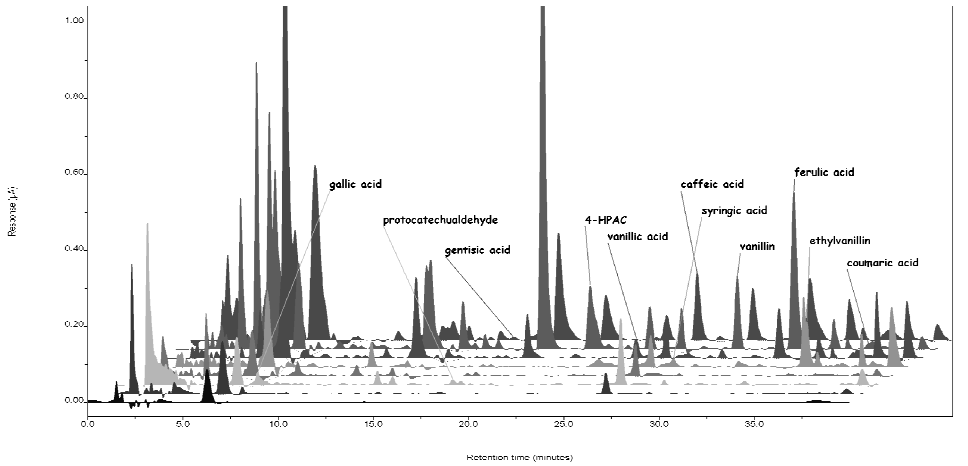

Fig. 5. Chromatogram of mead (Sváteční medovina bylinná, Medex), Condition: Column – Gemini C18 110A (150 x 3 mm, 3µm), mobile phase – aqueous 5 mM ammonium acetate with acetonitrile under gradient program, flow rate 0.4 mL/min, column and cells temperature 35 °C, detection 250, 300, 400, 500, 600, 700, 800 and 900 mV.

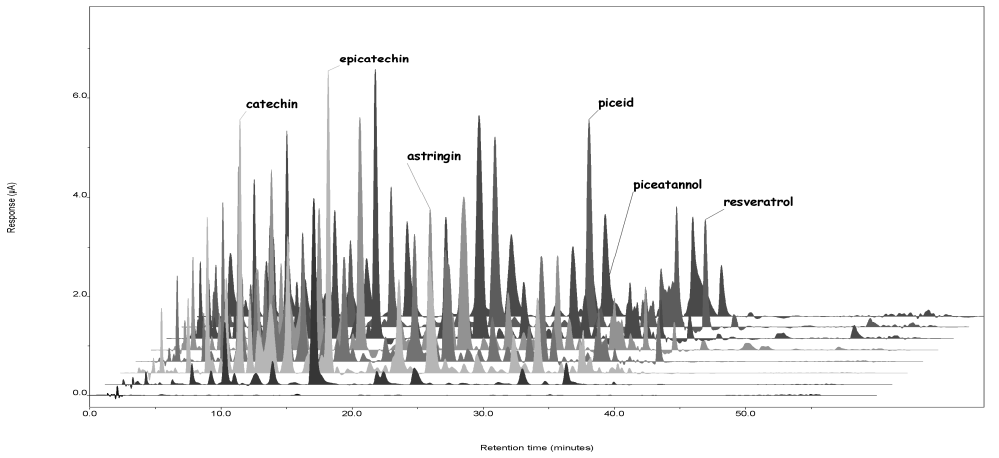

Fig. 6. Chromatogram of Japanese knotweed root´s extract, Condition: Column – Gemini C18 110A (150 x 3 mm, 3µm), mobile phase – aqueous 5 mM ammonium acetate with acetonitrile under gradient program, flow rate 0.4 mL/min, column and cells temperature 35 °C, detection 250, 300, 400, 500, 600, 700, 800 and 900 mV.

3.2. Method validation

Under the optimized experimental conditions, calibration curves of standard compounds were measured. Linear regression analysis using the least squares method was used to evaluate the calibration curve of each analyte as a function of concentration. The LODs were determined using lower concentrations of standards for a S/N of 3:1 (S/N = 3). Baseline noise was evaluated by injection of blank (mobile phase) in five replications. Similarly, the LOQs were calculated from a S/N of 10:1. LOD and LOQ values for target compounds are summarized in Table 1.

Table 1. Limits of detection (LOD) and limits of quantification (LOQ) of standard compounds.

Standard	*LOD (μg.L^{-1})*	*LOQ (μg.L^{-1})*
Gallic acid	*5.4*	*18.0*
Protocatechuic acid	*6.5*	*21.6*
p-hydroxybenzoic acid	*15.0*	*50.0*
Gentisic acid	*2.8*	*9.3*
Protocatechualdehyde	*4.8*	*16.1*
4-hydroxyphenylacetic acid	*4.2*	*13.9*
Vanillic acid	*5.3*	*17.6*
Caffeic acid	*4.4*	*14.8*
Chlorogenic acid	*3.5*	*11.6*
Syringic acid	*4.4*	*14.8*
Coumaric acid	*4.0*	*13.3*
Ferulic acid	*5.3*	*17.6*
Sinapic acid	*6.5*	*21.6*
Rutin	*25.0*	*83.3*
Vanillin	*2.2*	*7.3*
Ethylvanillin	*1.9*	*6.5*
Catechin	*4.2*	*14.0*
Epicatechin	*6.3*	*21.0*
Astringin	*25.1*	*83.6*
Piceid	*17.2*	*57.3*
Piceatannol	*18.0*	*60.0*
Resveratrol	*8.1*	*27.0*

3.3. Results of sample analysis

The target compounds in the extracts were identified using comparison with authentic standards. In addition to the retention times and hydrodynamic voltammograms, the ratios of the areas of the dominant, predominant, and the postdominant peaks, recorded at different applied potentials were used for peak identify confirmation. Further, selected compounds were quantified in all samples using external standard calibration method.

3.3.1 Wines

The content of target compounds was different in samples of white and red wine. It is possible to summarize that red wines contain more phenolic compounds and their higher amounts as well. In Table 2 are summarized the results obtained.

Table 2. Summary of substance contents in samples of wine.

Standard	*White wines*	*Red wines*
	substance content (mg.L^{-1})	*substance content (mg.L^{-1})*
Gallic acid	*0.610 – 4.013*	*7.364 – 27.353*
Protocatechuic acid	*0.346 – 1.688*	*1.869 – 4.628*
p-hydroxybenzoic acid	*0.266 – 2.301*	*3.122 – 4.954*
4-hydroxyphenylacetic acid	*0.117 – 0.457*	*0.185 – 1.248*
Vanillic acid	*0.299 – 1.933*	*9.990 – 19.208*
Caffeic acid	*0.173 – 1.565*	*1.213 – 4.843*
Chlorogenic acid	*0.112 – 2.075*	*0.574 – 4.887*
Syringic acid	*0.045 – 0.358*	*2.006 – 5.319*
Vanillin	*0.381 – 3.307*	*8.420 – 21.989*
Coumaric acid	*0.195 – 1.190*	*0.943 – 5.659*
Ferulic acid	*0.345 – 0.912*	*1.170 – 2.269*
Sinapic acid	*0.125 – 0.340*	*0.414 – 1.029*
Catechin	*0.199 – 2.513*	*7.518 – 15.654*
Epicatechin	*0.210 – 6.794*	*9.730 – 22.413*
Rutin	*0.875 – 5.078*	*7.699 – 13.323*
Resveratrol	*0.333 – 0.987*	*0.628 – 1.853*

3.3.2 Meads

The content of phenolic compounds differs in accordance with a type of mead. The meads with an addition of fruit juice contain higher amount of phenolic compounds in comparison with natural meads. An exception from this rule makes vanillin. Addition of fruit juice has no influence on final amount of vanillin. Moreover, vanillin is the substance which is frequently added into the mead of purpose to improve organoleptic properties. In two samples of meads the amounts 40.43 mg.L^{-1} and 65.04 mg.L^{-1} of vanillin were determined. These two values were extreme and averted from the all others values. Hence, these values were not included into Table 3, where all others results are summarized. In Table 3 are presented the results obtained from analyses of natural and fruit meads.

Table 3. Content of target compounds in samples of mead.

Standard	*Natural mead*	*Mead with fruit juice addition*
	substance content (mg.L^{-1})	*substance content (mg.L^{-1})*
Gallic acid	*0.092 – 0.316*	*2.589 – 3.367*
Protocatechuic acid	*0.053 – 0.346*	*0.986 – 1.525*
Gentisic acid	*0.022 – 0.109*	*0.175 – 0.191*
Protocatechualdehyde	*0.027 – 0.064*	*0.238 – 0.272*
4-hydroxyphenylacetic acid	*0.041 – 0.168*	*0.494 – 1.867*
Vanillic acid	*0.071 – 0.220*	*0.369 – 0.742*
Caffeic acid	*0.081 – 0.797*	*1.317 – 2.998*
Syringic acid	*0.035 – 0.271*	*1.532 – 2.443*
Vanillin	*0.570 – 5.171*	*1.088 – 1.548*
Ferulic acid	*0.079 – 0.771*	*1.701 – 2.556*
Ethylvanillin	*0.034 – 0.332*	*0.985 – 1.496*
Coumaric acid	*0.073 – 0.273*	*0.643 – 2.335*

3.3.3 Japanese knotweed

A number of groups of samples were analyzed in this work. The amounts of target compounds in extracts prepared from roots of this plant differ in dependence on variety, the way of plant growing, climate and others. However, the results achieved were summarized and in Table 4 are summed up most frequent ranges of substance contents.

Table 4. Summary of substance content in Japanese knotweed´s roots.

Standard	*Substance content (mg.g^{-1})*
Catechin	*1.18 – 2.15*
Epicatechin	*2.12 – 2.65*
Astringin	*1.09 – 2.48*
Piceid	*3.32 – 5.68*
Piceatannol	*0.01 – 0.04*
Resveratrol	*0.16 – 0.41*

4. Conclusions

Coulometric multichannel electrochemical detection is very selective and sensitive tool in sphere of liquid chromatography. It can provide superior sensitivity over the other detectors commonly used together with HPLC. The coulometric array detection would be ideal choice to separate the overlap peaks of the co-eluting analytes. In addition, due to slight influence of the matrix, the samples of beverages do not require any special pretreatment except dilution and filtration. These findings were confirmed also during our work with samples of beverages – wines and mead as well as with samples of plant extracts.

HPLC with CoulArray detection is very sensitive separation technique. It is very suitable for analysis of phenolic acids (derivatives of benzoic and cinnamic acids). The limits of detection of these compounds were in the range 2.8 – 15.0 $\mu g L^{-1}$. CoulArray detection turned out to be very sensitive for catechins and stilbenes, too.

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