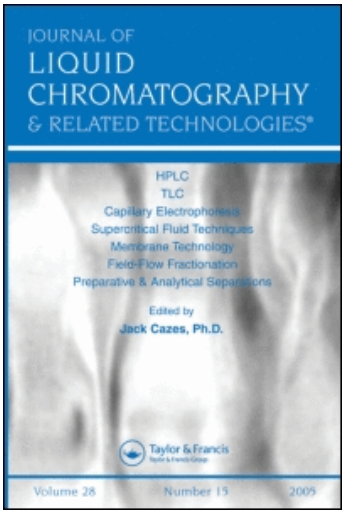

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Yongxin Zhu[a]; L. A. Coury[a]; Hong Long[a]; C. T. Duda[a]; Candice B. Kissinger[a]; Peter T. Kissinger[a]
[a] Bioanalytical Systems, Inc., West Lafayette, IN, USA

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LIQUID CHROMATOGRAPHY WITH MULTI-CHANNEL ELECTROCHEMICAL DETECTION FOR THE DETERMINATION OF RESVERATROL IN WINE, GRAPE JUICE, AND GRAPE SEED CAPSULES WITH AUTOMATED SOLID PHASE EXTRACTION

Yongxin Zhu, L. A. Coury, Hong Long, C. T. Duda,
Candice B. Kissinger, Peter T. Kissinger*

Bioanalytical Systems, Inc.
2701 Kent Avenue
West Lafayette, IN 47906, USA

ABSTRACT

A sensitive and selective liquid chromatography/ electrochemistry method with multi-channel detection was developed for the determination of the natural product trans-resveratrol in wines, grape juice, and grape seed capsules. Samples were prepared with an automated solid phase extraction workstation. Chromatographic separation was achieved on a C_{18} (100 x 2.0 mm) 3 μm column with a mobile phase containing 20 mM NaAc, 0.5 mM EDTA, pH 4.5, and 18 % acetonitrile at a flow rate of 0.4 mL/min. A four channel detector with glassy carbon electrodes was used, which can control up to four working electrodes simultaneously with applied potentials of +800, 700, 600, 500 mV vs. Ag/AgCl, and gave a better characterization of resveratrol in the complex matrices. The calibration curve was linear over the analytical range of 5-1000 ng/mL. With this method the content of resveratrol in different wines, grape juice and grape seed capsules was determined.

INTRODUCTION

Resveratrol (trans-3,5,4'-trihydroxystilbene) is a natural phenolic compound. It is found in many plants, such as mulberries, grapes,[1,2] and peanuts.[3] Both trans- and cis-resveratrol are found in red wine.[4] It has been reported that resveratrol may be effective in inhibiting platelet aggregation, altering eicosanoid synthesis and modulating lipoprotein metabolism.[5,6,7,8] Recent findings suggest that resveratrol is remarkably potent for preventing skin tumors in mice and at inhibiting the in vitro replication of human leukemia cells.[9,10] LC methods with UV or fluorimetric detection have been reported for the determination of resveratrol and resveratrol glucosides in grape berries and wines,[11,12,13,14] and in biological samples.[15,16,17,18] However, most published methods used slow gradient elution methods with these relatively nonselective detectors.

LC with electrochemical detection (LCEC) has been shown to be selective and sensitive for the determination of phenolic compounds in natural sources.[19,20,21] This paper describes an isocratic LC method with multi-channel electrochemical detection for the determination of resveratrol in wines, grape juice, and grape seed capsules with an automated solid phase extraction workstation for sample preparation.

EXPERIMENTAL

Apparatus

The LCEC system was composed of a chromatographic pump (PM-80, BAS, West Lafayette, IN, USA), a ODS 3 μm column (PEEK 100 x 2.0 mm, BAS), an experimental multi-channel amperometric detector (epsilon™, BAS) coupled to four glassy carbon working electrodes and referenced to a Ag/AgCl electrode. Data were acquired and integrated using BAS ChromGraph version 9.13 chromatography software. An autosampler (CMA/200, CMA, Stockholm, Sweden) with a 20 μL loop was used for sample injection. Automated solid phase extraction was performed using a Zymark RapidTrace workstation (Zymark, Hopkington, MA) equipped with two extraction modules, operating in parallel, using 100 mg of C18 sorbent contained in 1 mL solid-phase cartridges (Bond-Elut, Varian). The workstation was operated by a RapidTrace Controller using RapidTrace software (version 1.11).

Chemicals and Reagents

Trans-resveratrol was purchased from Sigma (St Louis, MO), cis-resvera-

trol was made from trans-resveratrol as reported by Goldberg.[4] Acetonitrile and methanol were of HPLC grade (Burdick & Jackson, Muskegon, MI), other reagents were of analytical grade and from Mallinckrodt (Paris, KY). Reagent grade water was prepared from in-house deionized water using a NANOpure system (Barnstead/Thermolyne, Dubuque, IA). Red wines and white wine were from California. Grape juice was purchased at a local supermarket. Grape seed capsules were from Meijer, Inc. (Grand Rapids, MI).

Preparation of Standard Stock Solution

Stock solution (1.0 mg/mL) of resveratrol was prepared in methanol. The solution was kept in the dark at 4°C when not in use.

Sample Preparation

A volume of 0.2 mL of wine or 1.0 mL of grape juice was transferred into 13 x 100 mm borosilicate glass tubes and diluted to 2.0 mL with water. For the grape seed capsules, to 0.1 g of powder was added 2.0 mL of methanol. The solution was sonicated for 15 min in the dark then centrifuged for 1 min. A 0.1 mL aliquot of the clear solution was transferred into a 13 x 100 mm borosilicate glass tube and diluted to 2.0 mL with water.

Sample tubes from above were placed in the Zymark RapidTrace workstation, programmed to process the samples by solid phase extraction. A procedure for the extraction of resveratrol from wine, grape juice, and grape seed was as outlined in Table 1. The eluents were collected into 12 x 75 mm borosilicate glass tubes. Aliquots (20 μL) were injected into the LC system by an autosampler.

Preparation of Calibration Curve and Recovery Test

A calibration curve was prepared for resveratrol by diluting the 1.0 mg/mL stock solution with methanol-water (1:1) to yield final concentrations of 5, 10, 50, 100, 500, 1000 ng/mL. A 20 μL volume was injected into the chromatograph.

Red wine, grape juice, and grape seed extracts (volumes as above) were spiked with 200, 100, and 200 ng of resveratrol, respectively. Each sample was treated as described under sample preparation. A 20 μL volume was then injected to determine the absolute recovery.

Table 1

Generalized Workstation Program Sequences for the Solid Phase Extraction of Resveratrol from Wine, Grape Juice, and Grape Seed

Step	Process	Reagent	Volume (mL)	Flow Rate (mL/min)
1	Wash cannula	H_2O	3	30
2	Precondition	CH_3OH	2	6
3	Precondition	H_2O	2	6
4	Load	Sample	2.1	1.8
5	Wash cartridge	H_2O	2	3
6	Wash cartridge	15% CH_3OH	1	3
7	Collect	CH_3OH	1	1.2
8	Collect	Air	0.4	10
9	Collect	H_2O	1	6
10	Collect	Air	0.4	10
11	Wash cannula	H_2O	3	30

RESULTS AND DISCUSSION

Extraction Development Using the Workstation

A procedure for the automated extraction of resveratrol from wine was developed, based on the RapidTrace program outlined in Table 1. The effect of experimental variables (sorbent selection, wash solvent, and elution solvent composition) that contributed most significantly to the selectivity and recovery of analyte was first evaluated by manual solid phase extraction. It was found that C18 cartridges yielded maximum selectivity and recovery. A concentration of 15% methanol proved to be the optimal wash solvent for eliminating possible polar interferences from extracts without eluting resveratrol.

A cartridge was loaded with a known amount of the resveratrol in water to confirm that no analyte was lost in any step of the procedure. The analyte was completely recovered when 100% methanol was used as the elution solvent. Finally, the sample was injected as a methanol-water (1:1) solution, as this resulted in both better resolution and sharper peaks.

Multi-Channel Electrochemical Detection

Until now most LC methods for the determination of resveratrol have used UV or fluorescence detection.[4] Multi-channel electrochemical detection has

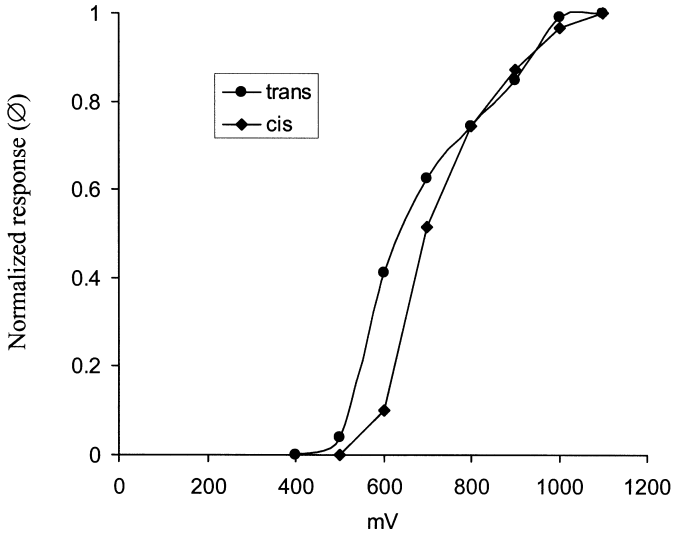

Figure 1. Hydrodynamic voltammograms of trans and cis-resveratrol in the mobile phase of choice at pH 4.5.

been useful in the identification and determination of antioxidants.[19] Figure 1 shows the hydrodynamic voltammograms of trans- and cis-resveratrol in the mobile phase (pH 4.5).

Four electrode detector experiments were performed at applied potentials of +500, 600, 700, and 800 mV. A depiction of the working electrode configuration of the four-channel electrochemical detector is shown in Figure 2. Using a radial flow pattern, an equal concentration of analyte passes over the four electrodes in the thin-layer channel. Chromatograms obtained from the four channels are shown in Figure 3. Simultaneously monitoring four potentials gives a better voltammetric characterization of trans- and cis-resveratrol in different natural sources, assuring peak purity by comparing ratios at different energies for both standards and samples.

Analytical Method Development

An isocratic reversed phase separation has been developed for trans- and cis- resveratrol using a mobile phase containing 20 mM NaAc, 0.5 mM EDTA, pH 4.5 and 18 % of acetonitrile. Figure 4 shows chromatograms at + 700 mV

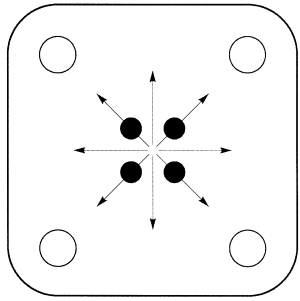

Figure 2. A depiction of the working electrode configuration for four-channel radial flow electrochemical detection (working electrode diameter = 2 mm).

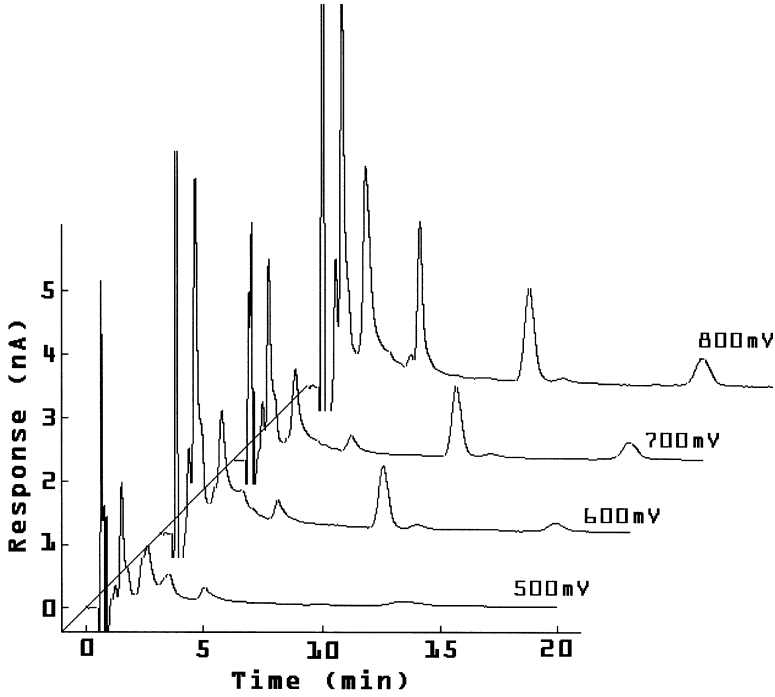

Figure 3. Chromatograms obtained from four-channel electrochemical detection.

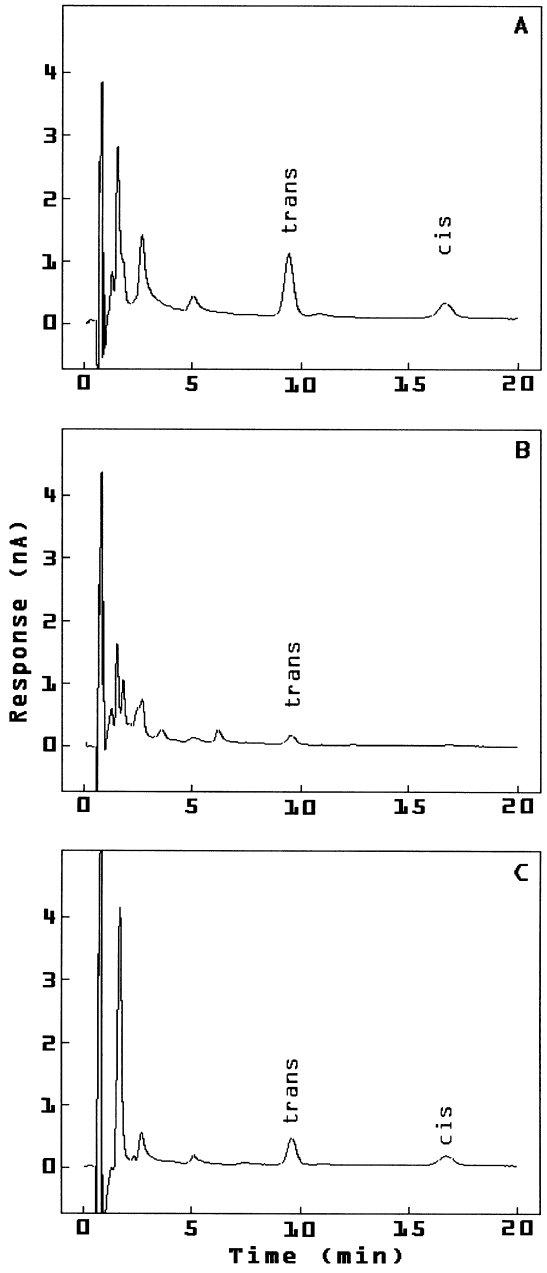

Figure 4. Chromatogram of extracts from red wine (A), grape juice (B) and grape seed capsules (C) (chromatographic conditions as described in text).

Table 2

Recovery of Resveratrol from Wine, Grape Juice, and Grape Seed

Sample	Added (ng/mL)	Found (ng/mL)	Recovery (%) Mean ± S.D.*	R.S.D. (%)	Error (%)
Red wine	200	204	102 ± 2.5	2.4	2.0
Grape juice	100	96.2	96.2 ± 4.2	4.3	3.8
Grape seed	200	194	97.0 ± 1.9	2.0	3.0

* n = 3.

for red wine (A), grape juice (B), and grape seed capsules (C), after solid phase extraction.

Calibration and Recovery

Calibration was performed over the range of 5 – 1000 ng/mL. The calibration curve was calculated by linear regression of the peak heights versus concentrations. The regression equation was Y = 258 x - 18 with correlation coefficient $r^2 = 0.9999$.

The recovery of known resveratrol concentrations added to the wine, grape juice, and grape seed capsule was determined. Recovery (Table 2) varied between 96-102 %.

Table 3

Concentration of Resveratrol in Wines, Grape Juice, and Grape Seed

Samples	Content (mg/mL)
Red wine 1	1.37
Red wine 2	0.32
White wine	0.04
Grape juice	0.05
Grape seed capsule	0.02*

* mg/g.

Quantitative Determination of Resveratrol

The concentration of resveratrol in red and white wines, grape juice, and grape seed capsules was determined by comparison with a standard solution (Table 3). It can be seen that the red wine contains more resveratrol than white wine. The values of resveratrol determined in the red wine are very close to those reported by an earlier LC-UV method,[4] but as with most natural products, variation based on the grape variety and processing of the vineyard are expected.

CONCLUSION

It has been demonstrated that liquid chromatography/electrochemistry with multi-channel electrode detection combined with automated solid phase extraction is suitable for the determination of resveratrol in different natural products.

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