CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Classification Of Flavonoids In Beverages

Purpose
Detection and classification of proanthocyanidins, flavonols, and anthocyanidins in wine and grape juice samples by liquid chromatography with UV, VIS and EC detection. Such a scheme could be useful for quality control in the beverage industry.

Existing Methods
Preparative chromatography followed by UV-VIS spectroscopy and the use of specific reagents to determine hydroxyl substitutions. This procedure is unsatisfactory because of the time and amount of analyte needed.

Reference
Structural Classification of Flavonoids in Beverages by Liquid Chromatography with Ultraviolet-Visible and Electrochemical Detection, S.M. Lunte, J. Chromatogr., 384 (1987) 371-382.

Conditions
Detector: BAS Dual 4B/17
Electrode: BAS dual series glassy carbon
Potential: upstream: +1.00 V, downstream 0.00 V, vs Ag/AgCl
Column: 5 µm, C-18 (250 X 4.6 mm) temperature controlled at 35°C
Guard Column: 5 µm, C-18
Mobile Phase: A: 0.05 M ammonium phosphate buffer (pH 2.5), B: acetonitrile. Linear gradient 5-25% B over 50 min. Mobile phase was sparged with helium throughout analysis. Flow rate was 1.5 mL/min.

Sample Preparation
The sample was loaded onto a disposable C-18 cartridge. Unwanted components were eluted with water and ammonium hydroxide. Flavonoids were eluted with methanol and diluted with mobile phase. (Some samples were injected without this cleanup step.)

Notes
The BAS 100 Electrochemical Analyzer was used to obtain cyclic voltammograms of standard flavonoids.

Proanthocyanidins absorbed maximally at 280 nm. Flavonols absorbed maximally at 280 and 360 nm. Anthocyanidins absorbed maximally at 525 nm. Catechol-substituted flavonoids were recognized by their high collection efficiencies (detection at downstream relative to upstream electrode). Typical results are presented in T1.

The characterization of flavonoids presented here can be duplicated with the BAS 200 Problem Solver, with its built-in oxygen removing capabilities and a 4-channel multi-wavelength detector option.

Dr. Susan Lunte is a Purdue Ph.D. in Bioanalytical Chemistry. This work was done at Procter and Gamble. Sue now works at the Center for Bioanalytical Research (CBAR) at the University of Kansas.

The information in this publication is supplied as a service to our customers. Performance of the methodology has not necessarily been verified by BAS technical staff.

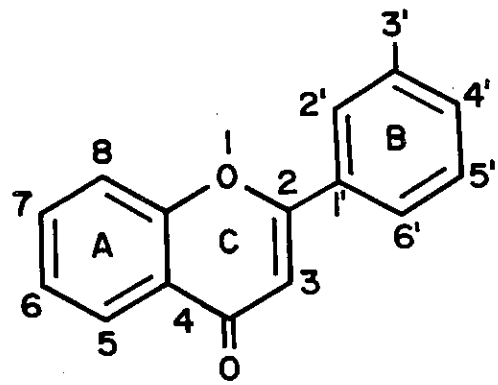

Figure 1. 2-phenylbenzopyrone, a typical flavonoid.

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Table 1. Probable identities of compounds.

FLAVONOIDS IN WINE

Peak No.	t_R(min)	UV-Max(nm)	UV-Max(nm)	Collection Efficiency (%)	Identity
A	19.44	280		13.4	Catechin
B	22.46	280		1.1	Procyanidin
C	23.00	280		33.0	Caffeic acid
D	23.93	280		10.4	Procyanidin
E	24.63	280		1.9	Prodelphinidin
F	25.37	280		15.9	Epicatechin
G	27.39	280		10.1	Procyanidin
H	28.22	280		12.8	Procyanidin
I	29.21	280		14.3	Procyanidin
J	30.59	310	525	10.0	Anthocyanidin*
K	34.70	280	360	31.0	Quercetin glycoside
L	35.75	280	360	3.7	Myricetin or kaempferol glycoside
M	39.93	280	360	3.9	Myricetin

*Delphinidin-ρ-coumaryl glycoside

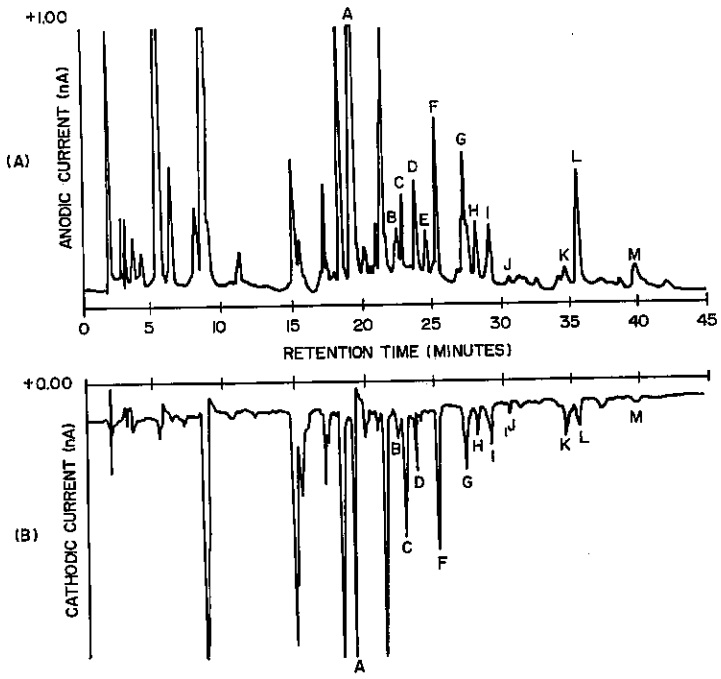

Figure 2. Series dual-electrode chromatogram of wine sample.