Evaluation of Detection Methods for the HPLC Determination of Stilbenes and Flavonoids in Red Wine

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Abstract

Several clinical studies have demonstrated that primarily alcohol and phenolic compounds such as trans-resveratrol are responsible for the health benefits of wine [1,2]. Therefore most research has been focused on the determination of trans-resveratrol since the initial publication has reported the presence of this phenol in commercial wines [3]. In newer publications also the cis-isomer of resveratrol and some flavonoids like quercetin are mentioned for their health benefit effects [4].
The aim of this study was to evaluate different detection methods such as UV, FLD and MS for the contemporaneant determination of stilbenes and flavonoids in red wine.
Measurements were achieved by HPLC. With a Hypersil BDS C18 column, a H_2O-MeOH-THF-H_3PO_4 gradient and 50°C good separation of the compounds of interest could be achieved. Using UV-absorbance detection at 320 nm for resveratrol and 377 nm for the flavonoids the plots of peak area versus concentration for all standardsolutions showed good linearity ($r^2 \geq 0,999$).
For the determination of trans-resveratrol also fluorescence detection was used (λ_{ex}=330nm, λ_{em}=374nm). Although standard solutions showed good linearity ($r^2 > 0,999$), the quantification of resveratrol in wine samples was difficult for the coelution of a fluorescent compound.
For the HPLC-MS measurements a different system was established. A Phenomenex Luna C18(2) was utilized as stationary phase, a Phenomenex ODS, C18, as guard column. To get good ionisation solvents were prepared without phosphoric acid, and to reduce the surface tension 15 µL/min acetonitrile were added to the eluent after the column through a tee. Collision induced dissociation was used for identifying the phenolic structures by their characteristic fragmentation pathways.

SUBSTANCE	DETECTION METHOD	WAVELENGTH	CALIBRATION CURVE	R^2	LOD [ng]
resveratrol	UV-visible	320 nm	y=127882 x - 23862	0,9999	1,312
	Fluorescence	λ_{ex}=330 nm λ_{em}=374 nm	y=239194 x + 4255,3	0,9996	0,700
	MS	m/z = 227,4	y=9e+06 x	0,9878	1,312 (full scan)
myricetin	UV-visible	377 nm	y=83581 x – 16071	1	2,775
	MS	m/z = 317,4	y=2e+07 x	0,9928	1,387 (full scan)
quercetin	UV-visible	377 nm	y=86972 x - 17345	0,9999	1,191
	MS	m/z = 301,3	y=3e+07 x	0,9910	0,397 (full scan)
kaempferol	UV-visible	377 nm	y=80444 x -10752	0,9999	0,85
	MS	M/z = 285,4	y=4e+07 x	0,9897	0,142 (full scan)

Tab.1
Calibration curves, statistical factors, limits of detection from resveratrol, myricetin, quercetin and kaempferol

References:

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2. S.Renaud and M.DeLorgeril; Lancet, 339 (1992) 49
3. E.H. Siemann and L.L.Creasy; Am.J.Enol.Vitic., 43 (1992) 49
4. A.I. Romero - Perez, R.M. Lamuela - Raventòs, A.L.Waterhouse, M.C.de la Torre-Boronat; J.Agric. Food Chem.; 44 (1996) 2124

Determination of polyphenolic compounds by UV-detection

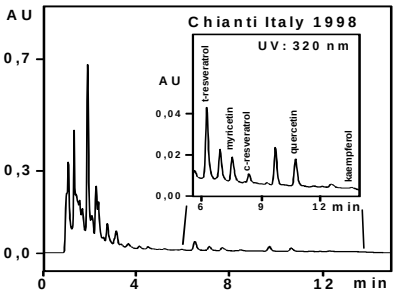

Figure 1
Hypersil BDS column C18, 125 x 4 mm, 3 µm; precolumn Hypersil BDS C18, 5 x 4 mm, 3 µm; gradient elution: solvent A: water, methanol, phosphoric acid; solvent B: water, methanol, THF, phosphoric acid; temp.: 50°C

Determination of polyphenolic compounds by fluorescence detection

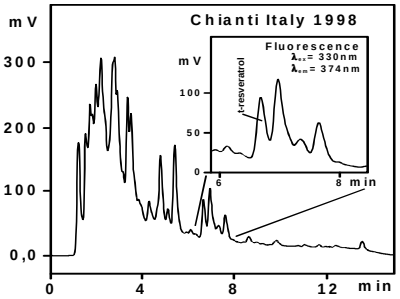

Figure 2
System as figure 1

Comparison of different detection methods

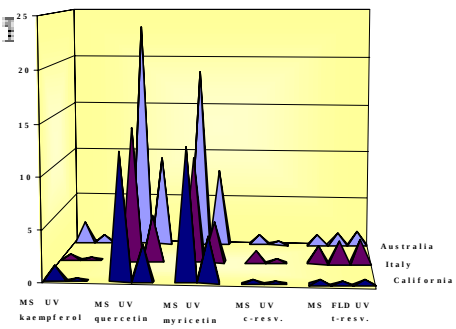

Figure 3
Cabernet from Australia and California, Chianti form Italy; MS = mass spectrometric detection, FLD = fluorescence detection

Determination of stilbenes and flavonoids by LC-ESI-MS

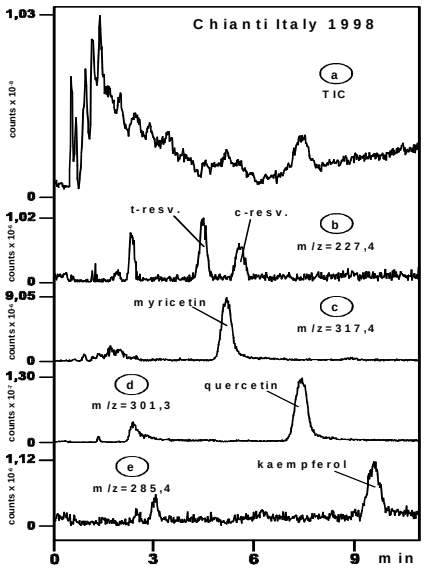

Figure 4
Column: Phenomenex Luna C18(2), 50 x 2mm, 3µm; precolumn: Phenomenex 4 x 4mm, 5µm; gradient elution: Solvent A: water, methanol; solvent B: water, methanol, THF; ACN by postcolumn liquid junction 15µL/min; 60°C

Results and Discussion

After optimizing the separation of cis- and trans-resveratrol, myricetin, quercetin and kaempferol by RP-HPLC, stilbenes and flavonoids were detected simultaneously by UV- and fluorescence detection (fig.1,2). Although UV-detection showed good baseline separation for the peaks of interest (fig.1), fluorescence detection didn`t (fig.2). Further investigations with HPLC-MS were carried out (fig.4) and compared to the already used detection techniques (fig.3).
The comparison pointed out, that each of these detection methods could be used for the quantification of trans-resveratrol, but not for the detection of the other structures. For them the mass spectrometer showed to be the best choise. As expected in the most cases the lowest detection limit could be achieved by LC-MS, only the detection of resveratrol by fluorescence showed to be better than the MS in the full scan mode (Tab.1).
Finally the HPLC-MS method gives the possibility of a rapid identification and quantification of polyphenols and glycosides even in complex sample matrices.