# Rogue Catalysts, Antioxidants, Paramagnetic Ions, EPR and HPLC:

# A New Protocol Needed?

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HPLC (High Performance Liquid Chromatogaphy) and EPR (Electron Paramagnetic Resonance) do not detect the same things: HPLC does not detect paramagnetic ions, which may well be catalytic and/or antioxidant in action. For example, a '99% pure' sample (by HPLC) of Fisetin contained paramagnetic ions, thus the antioxidant efficiency results for it could not be trusted. The dietary supplement Promensil, containing phyto-estrogens, also turned out to contain $Cu^{2+}$, of which the manufacturers were unaware. We suggest that EPR should be used in purity checks as a matter of protocol.

## 1. Introduction

In 2007, a sample of the polyphenol Fisetin ( found in strawberries, and related to Quercitin, found in wines) was purchased, for EPR and antioxidant efficiency measurements. According to HPLC, it was '99% pure', but turned out to contain a number of paramagnetic ions, as well as the expected free radical associated with (antioxidant) polyphenols. Since the ions can be catalytic and/or antioxidant or even pro-oxidant in action [1], the results of the antioxidant efficiency test could not be trusted. The EPR spectrum is shown in fig. 1, taken from [2].

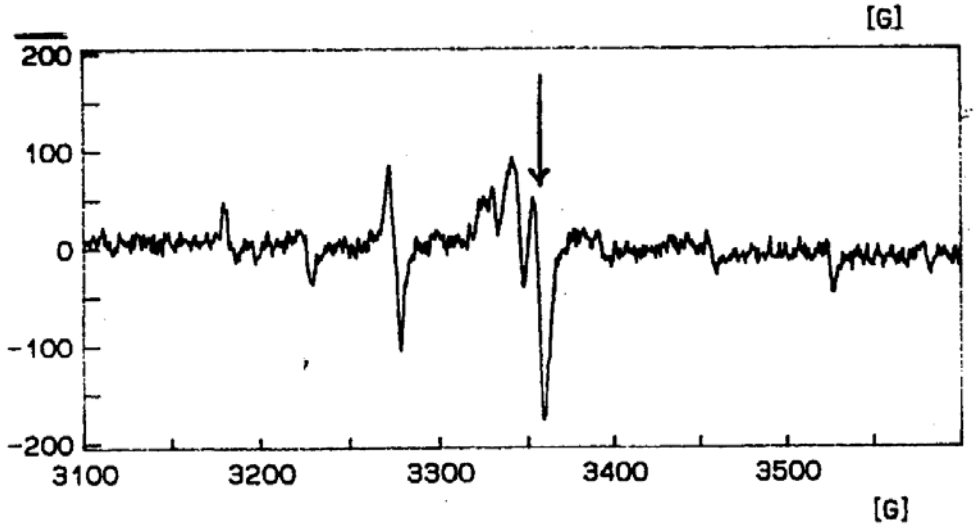


**Figure 1 EPR Spectrum of Fisetin**

A Fisetin sample without paramagnetic ions was expected to show a single sharp line from the phenolic free radical at the position indicated by the vertical bar: instead there are many lines and even the hint of a Mn spectrum. The free radical signal was present, and easily identified by its saturation behaviour, very different from that of the paramagnetic ions [2]. An EPR study of the dietary supplement Promensil, which contains phyto-estrogens

(polyphenols) showed that it contained $Cu^{2+}$, of which the manufacturers were unaware. The spectrum is shown in fig.2, and is that of a Cu porphyrin [3]. The manufacturers presumably

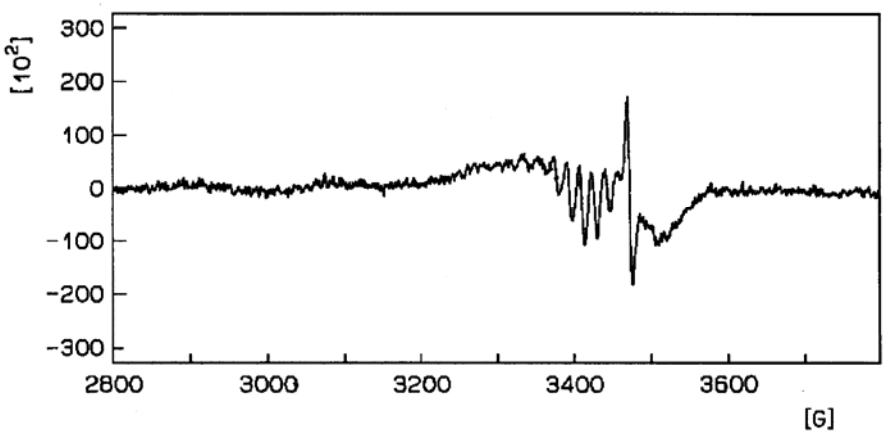


**Figure 2 EPR Spectrum of Promensil**

used HPLC to determine the phyto-estrogen content. In solution, CuII is known to form complexes with phenolate ions [4]. Further, if extracts of polyphenols are made in certain ways, and CuII is present, it will accompany the extracted polyphenols. We discovered this earlier while performing EPR on a 'pure'polyphenol extract from Shiraz grapeseeds [5]. A polyphenol extract from Promensil made in exactly the same fashion (see **Appendix**) gave an unchanged Cu porphyrin EPR spectrum. These examples demonstrate the difficulties involved in certifying chemicals as 'pure' using HPLC , when they are not tested for paramagnetic ions which may affect the phenomenon being studied.

## 2. Sample preparation

In each of the two cases above, the samples were in the solid state at ~20 C when being studied by EPR. Each sample was placed in its own special 2mm internal diameter quartz tube (Wilmad). The EPR spectrometer was a Bruker, operating at ~9.4 GHz.

## 3. Discussion

Buettner and Jurkiewicz [1] have discussed the role of 'catalytic metals', mainly the paramagnetic and redox ferrous and ferric ions, with regard to their effects on the antioxidant action of vitamin C, which can be spectacular in changing results expected when no such ions are present. *Mutatis mutandis*, surely other paramagnetic ions may well affect the antioxidant action of other molecules, such as polyphenols!

Do the purveyors of Promensil know how the presence of the Cu porphyrin affects the action of the 4 polyphenols it contains? When informed, their only comment, other than that they did not know of its presence, was that the Cu concentration was checked for, and found to be in the safe level for human ingestion. Promensil still contains the same amount of Cu porphyrin now as it did some years ago, when they were informed about it. Dark chocolate contains similar polyphenols to red wine, and also contains CuII , but the CuII is not mentioned when the good effects of dark chocolate on the human blood circulatory system are ascribed to the polyphenols!

We therefore suggest that, when polyphenols or other antioxidants are described as '99% pure' they should also be tested for paramagnetic ions, so that they can be described as having 'no paramagnetic ions detectable'. We further suggest that the effects of the paramagnetic ions present in systems known to contain them should be determined by suitable experiments. We have done the reverse in checking the effects of the paramagnetic

ions (mainly Cu and Fe) on the antioxidant effect of whiskies, which contain phenolics from being aged in oak. The removal of the phenolics, using polyvinylpolypyrollidine (PVPP), had only a small effect on the antioxidant efficiency [5].

**Acknowledgements**

Prof. Geoff Scollary, of Charles Sturt University, Wagga Wagga School of Wine Science and Viticulture, told us of the paper by Buettner and Jurkiewicz. Prof Peter Junk, Monash University School of Chemistry, gave us much useful information on the interactions of Cu with polyphenols. To them, our most grateful thanks.

**References**

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**Appendix**

The method of polyphenol extraction mentioned [5] is given below.

'For polyphenol analysis' the sample 'was extracted in 50mL 2:1acetone:water at 20 C for 24 hr. Extracts were filtered (Millipore #1 filters), acetone removed under reduced pressure at 35 C, and residual aqueous solution diluted to 25 mL with distilled-deionised water. Ten mL of this solution was transferred to a sample tube, frozen, and then freeze-dried. The solid residue was weighed and then dissolved in methanol (1 ml methanol/10 mg solid).'