Goldberg DM, Yan J, Ng E, *Diamandis EP*, Karumanchiri A, Waterhourse AL, Soleas GJ. A definitive gaschromatographic mass-spectrometric assay for resveratrol suitable for industrial and biological samples. **Clin Biochem** 1993;26:126-127.

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A definitive gas-chromatographic mass-spectrometric assay for resveratrol suitable for industrial and biological samples

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Resveratrol, a trihydroxy stilbene, is an important natural fungicide found in some grapes and other plants, synthesis of which is enhanced several orders of magnitude in response to infection. It is present in plant roots used in Japanese folk-medicine for treatment of many ailments including heart disease, hyperlipidemia and allergic reactions. A limited number of experiments in whole rats, guinea pig trachea, and cultured white blood cells have shown that it modulates hepatic VLDL synthesis, platelet coagulation, eicosanoid production and histamine release. Epidemiological evidence strongly suggests that wine-drinking countries have a lower incidence of death from myocardial infarction despite high rates of prevalence for established heart disease risk-factors. It has recently been suggested that resveratrol may be the constituent in wine that confers this protection. An obstacle to current research is the technical difficulties in current assays for resveratrol which require large sample sizes (50-500 mL), prolonged extractions, and a final set of chromatographic separations that do not easily separate the natural trans form from the cis form to which it is spontaneously converted by ultraviolet light.

Using as standard a preparation of resveratrol synthesized from appropriately substituted phenols by means of a Wittig reaction and verified as >95% pure by spectral analysis, a gas-chromatographic mass-spectrometric (GC-MS) assay was developed. This uses the Hewlett Packard GC Model 5890 with quadrupole MS Detector (Model 5970) coupled through a DB-5 column, 30 m long, 0.25 mm internal diameter and 0.25 μm thickness at initial and final oven temperature of 290°C and 315°C respectively at 10°C/min and 5-min hold, total assay time being <20 min per sample. The molecular ion is detected and quantitated at a mass of 228, with qualifier ions at 227 and 229.

Prior to GC-MS analysis, samples (1 mL) were passed through a C-18 SPE cartridge (Supelco) preconditioned with ethyl acetate, 96% (v/v) ethanol and 10% (v/v) ethanol in sequence. Resveratrol was eluted with ethyl acetate and 1 mL was collected of which 1 μL was injected. For samples with very low concentration, the eluate was dried and reconstituted in 200 μL ethyl acetate. The method gave the following performance characteristics: sensitivity 10 pg on injection or 10 p.p.b. in sample; CV within-batch 1.7%-5.7%; CV day-to-day <8%; quantitative recovery (~100%); linearity 10-40 p.p.b. for human urine and 60-300 p.p.b. for human serum. The method should have wide application in clinical, pharmacological and industrial investigations and is the first step in a program to develop formulations containing resveratrol (food, wine or pharmaceuticals) for human consumption.

Supported by the National Research Council and Andres Wines Ltd.