

National Bureau of Standards Certificate of Analysis Standard Reference Material 925 4-Hydroxy-3-methoxy-DL-mandelic Acid (VMA)

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This Standard Reference Material is certified as a chemical of known purity. It is intended primarily for use in the calibration and standardization of procedures employed in clinical analysis and for routine critical evaluation of the daily working standards used in these procedures.

Purity	99.4	percent
Uncharacterized Compound	maximum 0.5	percent
Keto-VMA	maximum 0.1	percent
Volatile Matter.	0.02	percent
Ash	0.004	percent

The value of purity has an estimated inaccuracy of 0.4 percent. The VMA for this Standard Reference Material was prepared at the National Bureau of Standards. Analyses were performed by A. Cohen, B. Coxon, E. R. Deardorff, R. A. Durst, D. P. Enagonio, A. J. Fatiadi, J. E. Fearn, B. Greifer, G. Marinenko, W. P. Schmidt, and J. K. Taylor of the Analytical Chemistry Division.

The overall direction and coordination of technical measurements leading to certification were under the chairmanship of R. Schaffer.

The technical and support aspects concerning the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by T. W. Mears.

Washington, D. C. 20234
May 24, 1973

J. Paul Cali, Chief
Office of Standard Reference Materials

The Standard Reference Material, dried to constant weight in a vacuum desiccator over concentrated sulfuric acid, showed little tendency to gain weight when exposed to laboratory air for 24 h. Losses in weight on heating for 1 h at 56 °C at a vacuum of 0.1 mm Hg (13 Pa) averaged 0.017 percent, with little additional loss on further heating (1 h). Prolonged heating (8 h) caused gradual decomposition.

The ash content was measured using 1.0-g samples.

Solutions of VMA exposed to light and air are unstable: originally colorless solutions turn pale yellow on standing. The initial photo-oxidation product is keto-VMA (4-hydroxy-3-methoxybenzeneglyoxylic acid). A procedure for the preparation of the previously unreported keto-VMA will be published. In acetone the rate of decomposition of VMA was found by spectrophotometry at 335 nm to be about 1 percent per day. Solutions of VMA in water, ethyl acetate, or 0.01 mol/liter hydrochloric acid did not change appreciably (< 0.5 percent) during storage for three days in the dark at room temperature, or when refrigerated for three months at 3-5 °C. Instability of VMA in solutions exposed to air was found to be in the order acetone > water > ethanol > methanol > ethyl acetate.

Analysis of the Standard Reference Material (SRM) by thin layer chromatography (tlc) revealed two impurities. When 0.5mg of this SRM was applied as a spot on non-activated silica gel G plates, developed with 4:4:1 (v:v:v) chloroform-acetic acid-water (solvent A) followed by spraying with 3 mol/liter sulfuric acid in methanol and heated for 2 min. in an oven at 110 °C showed VMA at R_f 0.63 and keto-VMA at R_f 0.92. The amount of keto-VMA detected was equivalent to 0.5 - 1.0 µg of the pure keto-VMA used as a standard. However, at least some of the keto-VMA is a chromatographic artifact. Two-dimensional tlc revealed keto-VMA in two locations, originating respectively from the positions that the VMA and the keto-VMA had occupied after the initial direction of chromatography. Furthermore, tlc of 0.1-mg quantities of keto-VMA with solvent A, followed by sulfuric acid charring, revealed the presence of a small proportion of vanillin, possibly as an artifact due to the instability of the keto-VMA in this chromatography. The second impurity which is as yet uncharacterized, was revealed at R_f 0.19 by tlc of the SRM on silica gel GF with solvent A followed by exposure to ammonia vapor and irradiation at 366 nm.

(VMA and keto-VMA are also detected by this technique.) With the silica gel GF and use of 1:1 (v:v) benzene-ethyl acetate or 5:4 (v:v) ethyl acetate-cyclohexane, the two impurities and the VMA were detected at R_F values similar to those given with solvent A. An attempt was made to isolate the uncharacterized impurity from 50 mg of SRM 925 by means of preparative thin-layer chromatography. However, this impurity underwent decomposition after being extracted and concentrated and was not detectable on chromatography by the previously employed technique. A definite proportion of the uncharacterized impurity in the SRM could not be assigned, but it is assumed to be less than 0.5 percent.

Phase solubility analyses of the SRM with 1:1 (v:v) benzene-ethyl acetate and with 5:4 (v:v) ethyl acetate-cyclohexane were performed by shaking mixtures for 8 days followed by gravimetric analysis of the solutions. The purity of the VMA appeared to be in the range 99.6-99.9 percent; however, tlc showed that both impurities remained in the solid phase. Thus, the results of these phase solubility analyses were not meaningful. In part this may be a consequence of the aforementioned instability of VMA.

Samples of VMA were titrated with standardized sodium hydroxide using a pH meter to the first inflection point of the titration curve. Complete titrations also were made in which both the carboxyl and phenolic groups were titrated. The pH values at the 0.5 and 1.5 equivalent points were 3.75 and 9.80, respectively, corresponding to the pK_1 and pK_2 for the compound. At the pH value 6.80, corresponding to the first inflection point, it was calculated that 99.91 percent of the carboxyl group is titrated; accordingly, the acid equivalent measured was increased by 0.09 percent. The sample contains a carboxylic acid equivalent of 99.83 percent.

The melting range (decomposition) of this SRM was 133-134 °C when measured in an open capillary tube heated at 1 °C per min.

Elemental analysis. Calculated for $C_9H_{10}O_5$ (198.17):
C = 54.54; H = 5.09; O = 40.37. Found: C = 54.6; H = 5.1;
O = 40.3.

This Standard Reference Material was prepared at the National Bureau of Standards by slowly adding glyoxylic acid to an ice-cold alkaline solution of guaiacol, with

mechanical stirring. The product was purified by successive recrystallizations from ethyl acetate-cyclohexane and 2-butanone-cyclohexane mixtures. (Details of this one-step synthesis of VMA will be published.)

The synthetic material was further characterized by gas-liquid chromatography (GLC) after per(trimethylsilylation) with TRI-SIL-Z and showed R_t 17.2 min. using a glass column (1.8 m x 2 mm i.d.) of Chromosorb Q (60-80 mesh) with 3 percent O-17 as liquid phase, isothermal at 150 °C; injection port at 180 °C, flame-ionization detector at 200 °C; nitrogen flow-rate at 25 ml per min; samples size 10 µg in acetone (1 µg/µl). Infrared spectrum maxima (KBr pellet). 1870, 1148 and 1715 cm^{-1} (C=O stretch). Nmr (methyl sulfoxide- d_6): singlet at δ 3.80 (-OCH₃), multiplet at δ 6.8 (aromatic protons), broad peak at δ 8.73 (-COOH). Ultraviolet spectrum: λ_{max} in 1.0 mol/liter K₂CO₃ (10-15 min after dissolution) at 251 nm (ϵ_M 1.022), 293 nm (0.429), and at 347 nm (0.001).

Note: The use of proprietary designations in this certificate are for information only, and should not be construed as an endorsement of the product by either the Department of Commerce or the National Bureau of Standards.