

Discussion

High-performance liquid chromatographic determination of urinary 2,5-hexanedione

A. Sturaro, G. Parvoli and L. Doretto

Ufficio Sicurezza e Prevenzione del CNR, Corso Stati Uniti 4, 35020 Padova (Italy)

(Received August 31st, 1992)

In a recent paper in this journal, entitled "Determination of total 2,5-hexanedione by reversed-phase high-performance liquid chromatography", Marchiseppe *et al.* [1] proposed a method for the HPLC of an *n*-hexane metabolite by using UV detection at 233 nm. Our more recent paper [2] demonstrated that such a wavelength was specific for 3-methylcyclopent-2-enone, an impurity often present in commercial 2,5-hexanedione. This contaminant was confirmed by comparing the gas and high-performance liquid chromatographic, mass spectrometric, infrared and ultraviolet data of the unknown compound with those of a synthesized reference sample.

Subsequently, in a paper entitled "Rapid and sensitive determination of urinary 2,5-hexanedione by reversed-phase high-performance liquid chromatography", Colombini *et al.* [3] proposed some valid modifications to the preparation of the sample, but, unfortunately used a wavelength similar to that of Marchiseppe *et al.* [1], which is unsuitable for identifying 2,5-hexanedione.

In Figs. 1 and 2 the UV and GC-MS analyses of Aldrich and Eastman Kodak 2,5-hexanediones are reported. The UV spectra of these two commercial 2,5-hexanediones (0.47 mg/ml), obtained by means

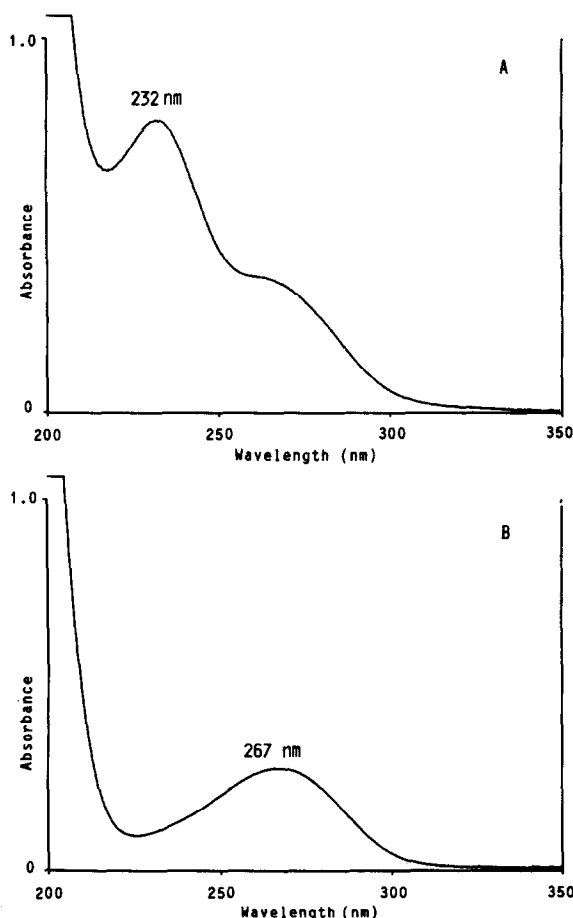


Fig. 1. UV spectra of 2,5-hexanedione from (A) Aldrich and (B) Eastman Kodak in water-acetonitrile (85:15), recorded from 200 to 350 nm with a Hitachi U-3200 spectrophotometer.

Correspondence to: A. Sturaro, Ufficio Sicurezza e Prevenzione del CNR, Corso Stati Uniti 4, 35020 Padova, Italy.

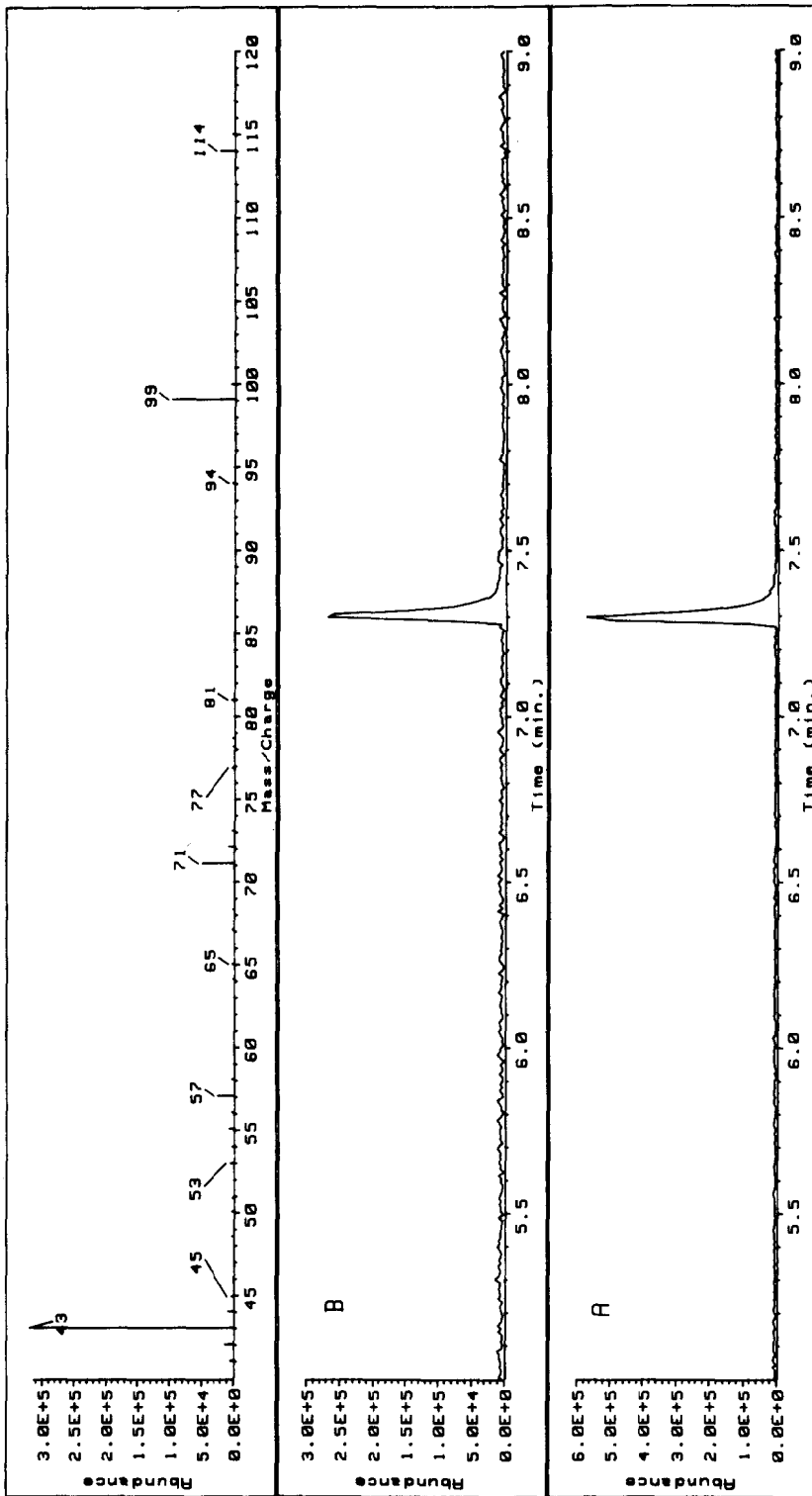


Fig. 2. GC-MS chromatograms and mass spectrum corresponding to the detected peaks in the analysis of 2,5-hexanedione samples from (A) Aldrich and (B) Eastman Kodak.

of a Hitachi U-3200 spectrophotometer in the same solvent as used by Colombini *et al.* [3], confirmed the definite difference due to the presence of the impurity, whereas under GC–MS conditions the same commercial samples (1.4 mg/ml) were identical. On the basis of the results, the maximum absorption wavelength of 2,5-hexanedione is 267 nm only [2,4].

In our opinion, in spiked urines 3-methylcyclopent-2-enone was introduced involuntarily as misleading interferent with 2,5-hexanedione. The impurity was thought to be the metabolite owing to the similar HPLC behaviour and high molar absorptivity [5]. In fact, this parameter greatly influences the response factor of the analyte in HPLC–UV determination and therefore a strong signal is associated with a small concentration of 3-methylcyclopent-2-enone owing to its high molar absorptivity, which is about 150 times higher than that of 2,5-hexanedione.

The presence of 3-methylcyclopent-2-enone in the urine of exposed workers may be attributed to the alkaline environment produced by sodium hydroxide and carbonate, which favours the cyclization of the present 2,5-hexanedione, according to the literature synthesis [5].

The GC–MS measurements were performed using a Hewlett-Packard Model 5890 gas chromatograph equipped with an Ultra-1 fused-silica capillary column (25 m × 0.2 mm I.D., 0.33 μm). The chromatographic system was coupled on-line with a Hewlett-Packard Model 5971 A quadrupole which recorded the electron ionization mass spectra by cyclically scanning from 40 to 250 mass units every 0.49 s. For GC separations the column temperature was programmed from 50°C (isothermal for 3 min) at 10°C/min to 150°C, maintained for 5 min. The injection volume was 1 μl under split conditions.

REFERENCES

- 1 I. Marchiseppe, M. Valentino, V. Stocchi and M. Governa, *J. Chromatogr.*, 495 (1989) 288.
- 2 A. Sturaro, G. Parvoli, S. Zanchetta, L. Doretti, G. Gori and G. B. Bartolucci, *J. Chromatogr.*, 590 (1992) 223.
- 3 M. P. Colombini, P. Carrai, R. Fuoco and C. Abete, *J. Chromatogr.*, 592 (1992) 255.
- 4 R. C. Weast and M. J. Astle (Editors), *CRC Handbook of Chemistry and Physics*, CRC Press, Boca Raton, FL, 59th ed., 1978, p. C-335.
- 5 R. M. Acheson and S. R. Robinson, *J. Chem. Soc.*, (1952) 1127.