Notes

A note on the chromatography of melatonin

The recent upsurge in research involving melatonin (N-acetyl-5-methoxytryptamine)[1,2] has led to an increase in the number of analytical procedures dealing with this compound[3–5]. The increased interest in this hormone stems from the fact that its release from the pineal gland appears to be under the direct control of the nervous system. In addition, the direct nervous connection between the pineal gland and the eye has led researchers to attempt to determine the relationship between this hormone and a biological clock. Recently[6] there has been a report of tumor cells that secrete this hormone. This report has serious implications due to the gonadal-inhibiting function of this compound. We have observed two points on the chromatographic behavior of melatonin that lead to improved results.

Ehrlich reagent[7] is a commonly used spray for the visualization of melatonin in thin-layer chromatography; however, the blue color develops rather slowly. We have

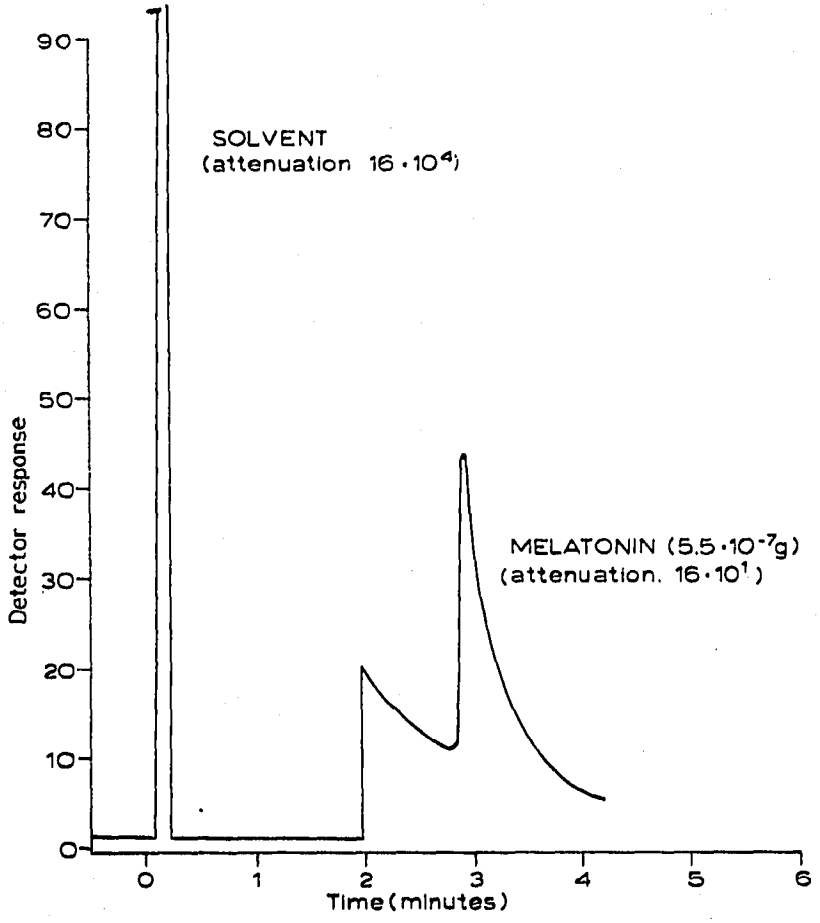

Fig. 1. Chromatogram of melatonin obtained under conditions given in text.

found that the exposure of the plates to long wavelength ultra-violet light following the Ehrlich spray gives much faster color development and an increase in sensitivity. Any source of long wavelength U.V. light held close to the plate surface may be used; however, the U.V. source must be removed following maximum color development because excess exposure leads to fading of the blue color.

The second point deals with the gas chromatographic separation of melatonin. This compound has been separated previously[3,4]. The latter reference used an all glass system for the analysis and reported a retention time of slightly over 10 min for melatonin. We have found that this compound can be readily chromatographed on a Silicone Oil DC-W98 column using an all metal system. The all metal system gives equal sensitivity, and the analysis time is approximately one third that of the best previously reported. In addition, a metal column is obviously cheaper and much more easily prepared.

A typical chromatogram of melatonin (Aldrich Chemical Co., Milwaukee, Wis. 53120) is shown in Fig. 1. The chromatogram was obtained using an F & M Model 5750 dual flame instrument. The operating conditions were as follows:

Column: 6 ft. by 1/8 in. O.D.
Solid support: 80/100 Chrom. P
Liquid phase: 10% Silicone Oil DC-W98
Carrier flow: 60 ml/min of He
Column temperature: 265°
Injection temperature: 250°
Detector temperature: 265°
Solvent: 95% ethanol
Hydrogen flow: 60 ml/min
Oxygen flow: 300 ml/min

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