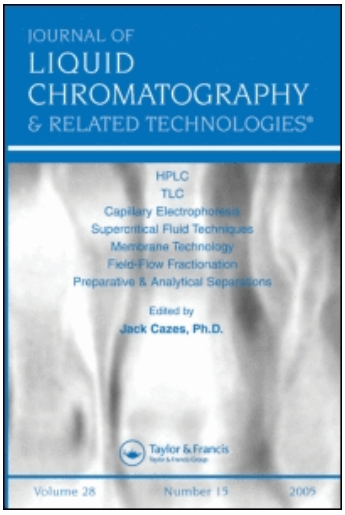

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Hassan Y. Aboul-Enein[a]; Suhair Abu-Zaid[a]
[a] Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC-03), King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

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Hassan Y. Aboul-Enein,* Suhair Abu-Zaid

Pharmaceutical Analysis Laboratory
Biological and Medical Research Department (MBC-03)
King Faisal Specialist Hospital and Research Centre
P.O. Box 3354
Riyadh 11211, Saudi Arabia

INTRODUCTION

Since its discovery nearly forty years ago, melatonin (N-acetyl-5-methoxy tryptamine) has been widely investigated with regard to the factors that control its synthesis and in reference to its endocrine consequences.[1] The pineal hormone melatonin is involved in the transduction of photoperiodic information which regulates a variety of physiological functions, including reproduction and body weight.[2-4] Recently, Wetterberg[5] published a review on the clinical applications of melatonin in humans and its role on traits such as sleep, Circadian rhythm, surgical stress, and anesthesia.

Also, age-related melatonin studies and its uses as a mediator for sleep disturbance in depression, for jet-lag, and as a skin protector for ultraviolet light were discussed. 2-[^{125}I]-iodomelatonin, a new radioligand with high specific activity, has been shown to label a high affinity melatonin binding site in the retina, which possesses the same pharmacological characteristics as the melatonin receptor-modulating calcium-dependent release of [^{3}H]dopamine.[6]

Duncan et al.[7] characterized a brain binding site using 2-[^{125}I]-iodomelatonin in the Syrian hamster, a species in which the photo periodic effects of melatonin are well established. It was found that 2-[^{125}I]-iodomelatonin is a potent and selec-

tive ligand to label melatonin binding sites in the hamster brain. In the present study, we describe an analytical method for 2-iodomelatonin, by a reversed-phase high performance liquid chromatography (HPLC) using a fluorescence detector, as well as studying its stability in the preparation of the radioactive iodomelatonin.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Water 501 solvent delivery pump, a Rheodyne model 7125 injector, and Water 470 scanning fluorescence detector, operated at λ excit 285 nm and λ emiss 345 nm. A Supelcosil LC-18 column (25 cm x 0.46 cm I.D., coated on silica gel of particle size 5 μm) was purchased from Sigma-Aldrich Company (St. Louis, MO, USA). A mobile phase consisted of ammonium acetate buffer solution 10 (mM) adjusted with glacial acetic acid to pH 4, methanol, acetonitrile (8:1:1 v/v) was used.

Chemicals

2-Iodomelatonin (2-IM) was purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Ammonium acetate and methanol, HPLC grade, were

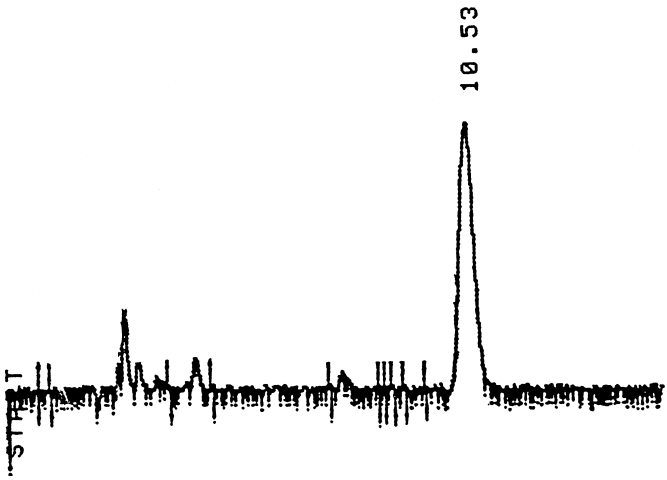

Figure 1. HPLC chromatogram of 2-iodomelatonin (conc. 0.4 pmole/μL).

obtained from Fisher (Springfield, NJ, USA). Glacial acetic acid was obtained from Sigma-Aldrich Company (St. Louis, MO, USA).

RESULTS AND DISCUSSION

2-Iodomelatonin was determined using fluorescence detector as shown in the chromatogram of Figure 1. The calibration curve showed linearity from 1 to 0.1 pmole/μL (r = 0.994) as shown in Figure 2. The limit of detection was 0.1 pmole/μL at a 3:1 signal-to-noise ratio. 2-Iodomelatonin solution was found to be stable for more than 60 days as shown in the chromatogram of Figure 3. The chromatogram shows an overloaded peak which belongs to 2-iodomelatonin, without showing any extensive degradation.

The results obtained are in good agreement with those of Vitale et al.[1] and Itoh et al.[8] This method is simple, sensitive, selective, and stability-indicating. It is suitable for quality control analysis for unlabelled 2-iodomelatonin which

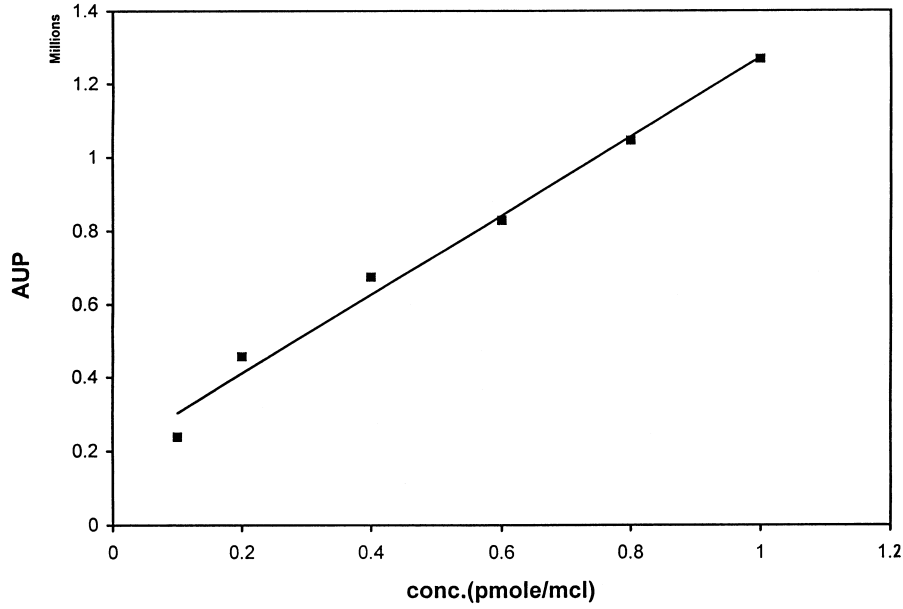

Figure 2. Calibration curve plotted between concentration (pmole/μL) and area under the peak (AUP).

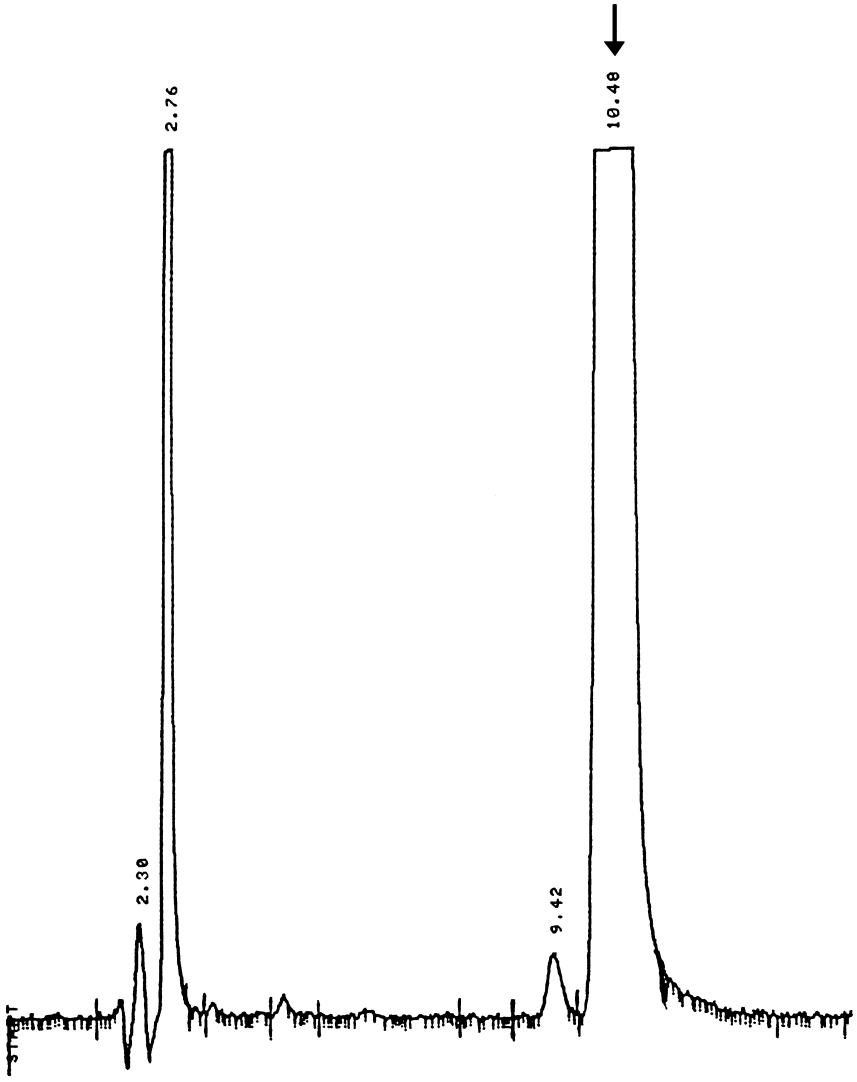

Figure 3. HPLC chromatogram shows the stability of 2-iodomelatonin solution after 60 days.

is the precursor for the preparation of radio-iodinated labelled I^{123}-iodomelatonin which is in progress.

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