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Short Communication

Melatonin: quantitative analysis in pharmaceutical oral dosage forms using thin-layer chromatography (TLC) densitometry

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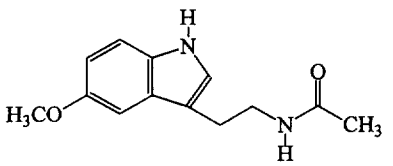
Abstract

A thin-layer chromatography densitometric method for the quantitative analysis of melatonin in tablets and capsules has been developed. High-performance thin-layer chromatography (HPTLC) silica gel plates were used as the stationary phase and the elution was made with CH_2Cl_2/MeOH (95:5). Densitometric evaluation of the spots was performed with a TLC scanner in reflectance mode at 223 nm with a deuterium lamp. A linear relation between the peak areas and the amount of melatonin deposited was found in the range 63–399 ng. The method was applied with success for the quantitative determination of melatonin in commercial and experimental samples.

Keywords: Melatonin; Densitometry; Thin-layer chromatography; Oral dosage forms

1. Introduction

Melatonin (*N*-[2-(5-methoxy-1*H*-indol-3-yl)ethyl]-acetamide) is a methoxyindole secreted by the pineal gland where its synthesis is controlled by external factors such as environmental light. Melatonin lightens skin cell pigment and suppresses ovarian functions [1]. Because of its possible role in biological rhythms, melatonin has been widely studied and has become popular in recent years.



melatonin

The pharmacological effects of melatonin have been investigated in psychiatric disorders, sleep disorders [2–6], Alzheimer disease [7], aging [8,9], and hypercholesterolemia [10,11].

The best results were obtained in the prevention of jet-lag syndrome [12–14] and in delayed sleep phase syndrome [15–17]. Moreover, recent studies reported that melatonin seems to be a hydroxyl radical scavenger [18,19]. In 1993 melatonin obtained USA orphan drug status with an orphan designation regarding treatment of circadian rhythm sleep disorders in blind people with no light perception [20]. The therapeutic use of melatonin in cancer therapy has mainly been investigated by Italian groups [21–24].

In the most recent scientific literature, the quantitative determination of melatonin in biological fluids was performed primarily by liquid chromatography [25–38] or radioimmunoassay [26,39–47]. Some methods such as ELISA [39,48], GC/MS [49–51], differential pulse voltammetry [52] and others were reported. Thin-layer chromatography (TLC) densitometry was employed for the determination of melatonin and other indolic compounds after derivatization with *p*-dimethylaminocinnamaldehyde or *o*-phthalaldehyde [53,54].

However, none of these methods was applied to pharmaceuticals. This paper describes a novel application of TLC densitometry for direct determination of melatonin in tablets and capsules without derivatizing agents.

The use of melatonin as a dietary supplement extends the number of possible associations with other substances. Therefore, the development of a chromatographic method was necessary to provide a general procedure applicable to a large number of preparations, even if containing excipients or other

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active substances that could interfere with spectrophotometric or fluorometric determinations.

Results indicate that the method developed can be used for quality control of preparations containing melatonin and thus may be suitable for monitoring uncontrolled products available in some countries.

2. Experimental

2.1. Chemicals and samples

Standard melatonin was obtained from Acros Organics (NJ, USA).

The solvents and other chemicals were obtained from Farmitalia Carlo Erba (Italy). The filters (0.45 μm) were obtained from Whatman (NJ, USA). The investigated products (tablets and capsules) were over-the-counter preparations available in the USA (dosage: 3 mg of melatonin; excipients: not reported) and experimental preparations manufactured by Stabilimento Chimico Farmaceutico Militare (Florence, Italy) with dosages of 10 and 20 mg of melatonin. The excipients of these experimental tablets were cellulose in microcrystalline form (55 and 45 mg, respectively), magnesium stearate (3 mg), and lactose (62 mg).

High-performance thin-layer chromatography (HPTLC) silica gel 60 F_{254} plates (10 × 10 cm), with a layer thickness of 0.20 mm, were obtained from Merck (Darmstadt, Germany).

2.2. Equipments

Camag Linomat IV was used as the application device. A horizontal-development chromatographic chamber and a TLC Scanner II were also provided by Camag (Muttenz, Switzerland). The scanner was combined with a Merck Hitachi integrator (Merck, Darmstadt, Germany).

3. TLC method

3.1. Preparation of the sample solutions

A quantity of powdered tablets or capsules, corresponding to 20 mg of melatonin, was suspended in 100 ml of ethanol and agitated for 20 min in an ultrasonic bath. The suspension was filtered through a membrane filter of 0.45 μm. The filtrate was diluted with ethanol to obtain a final concentration of 0.020 mg/ml.

3.2. Preparation of the standard solution

The standard solution was prepared through dissolution of 21.0 mg of melatonin in 100 ml of ethanol. Further dilution was necessary to obtain a final solution of 0.021 mg/ml.

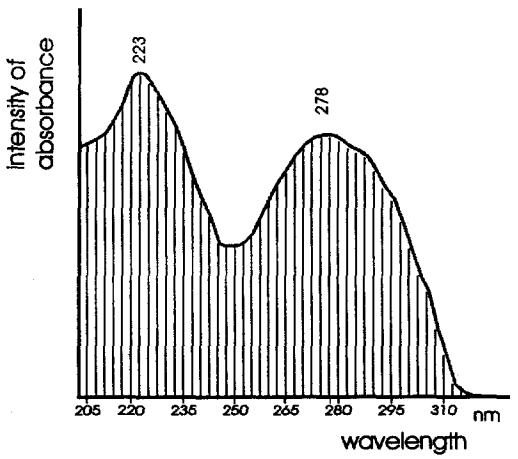

Fig. 1. Absorption spectra of melatonin (λ_{max} = 223 nm) on silica gel plate.

3.3. Chromatographic procedure

The HPTLC plates were prewashed with eluent CH_2Cl_2/CH_3OH (95:5), dried for 30 min in an oven at 100°C and cooled down in a desiccator.

Sample volumes (9 μl) and standard solutions (3–19 μl) were applied on two opposite sides of a plate in narrow bands using the CAMAG Linomat IV (spray-on technique with a Hamilton 100 μl microsyringe), starting at 10 mm from the sides of the plate.

The band length was 6 mm and the distance between the lanes was 10 mm.

The sample volumes were applied with a delivery rate of 15 s/μl and a nitrogen pressure of 2.5 bar. The Camag horizontal-development chamber (in the 'tank configuration') was equilibrated with the mobile phase CH_2Cl_2/CH_3OH (95:5) for one hour prior to use. The plate was developed at room temperature from both sides towards the middle. After development, the plate was dried in the air for 5 min and in an oven at 50°C for 10 min. Densitometric evaluation of the spots was performed with the scanner device.

The absorption spectrum of melatonin on silica gel was registered to confirm the UV maxima reported in the literature (Fig. 1). The spots were analysed at 223 nm with a deuterium lamp, using the following parameters: single-beam reflectance mode; monochromator bandwidth 10 nm (micro position); slit dimensions 0.3 × 3 mm; scanning speed 0.5 mm/s; automatic zeroing before each track; automatic sensitivity adjustment.

All the operations were conducted under normal environmental light. The absence of an appreciable degrading process in the operating conditions was verified.

4. Results and discussion

The results presented in this paper demonstrate that TLC densitometry can be applied to qualitative and quantitative analysis of melatonin in pharmaceutical dosage forms. A

Table 1
Assay results for the TLC densitometric determination of melatonin in experimental and commercial preparations. Volume of sample solutions applied: 9 μl. The results given are the means of six determinations

Preparation	Theoretical content of melatonin (mg)	Amount of melatonin found		
		$\bar{x}$ (mg)	RSD (%)	Found (%)
A (tablets)	10	10.09	1.9	100.9
B (tablets)	10	9.77	1.7	97.7
C (tablets)	20	20.46	1.7	102.3
D (tablets)	3	2.90	2.1	96.7
E (capsules)	3	2.95	2.0	98.3
F (tablets)	3	2.80	2.3	93.3

Preparations A–C were manufactured by Stabilimento Chimico Farmaceutico Militare. Preparation F contains vitamin B_6; in the chromatographic conditions a complete separation between vitamin B_6 and melatonin was found.

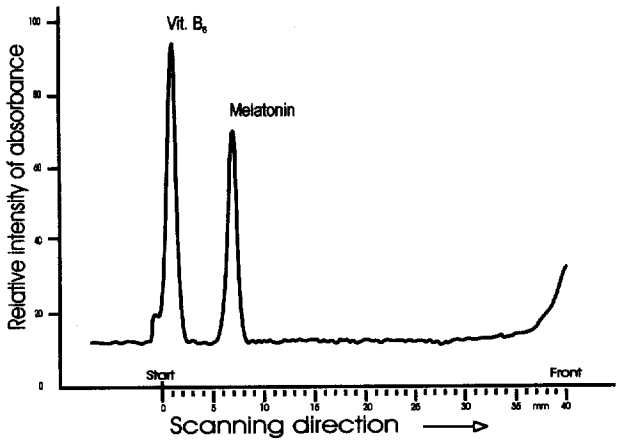

Fig. 2. Densitogram of a sample solution containing melatonin and vitamin B_6.

densitogram of the sample containing melatonin and vitamin B_6 (preparation F, Table 1) is presented in Fig. 2. The R_f value for melatonin was 0.17.

The relationship between the peak areas and the amounts of melatonin deposited was found to be linear in the range 63–399 ng. The least-squares regression equation, with the value for the corresponding correlation coefficient, was $y = 14.977x + 403.55$ ($r = 0.9981$), where the concentration of melatonin (x) was expressed in ng. The calibration points were obtained in triplicate at nine levels by applying 3–19 μl of the standard solution in steps of 2 μl (Fig. 3).

The precision of multiple scans of the same spot was found to be less than 1.0% relative standard deviation (RSD) at all levels of the calibration curve and the inter-assay precision (six replicates and three scanning runs) was found to be less than 2.3% RSD.

The limit of detection (LOD) and the limit of quantification (LOQ) were 18.4 and 61.3 ng, respectively.

Accuracy was assessed by spiking melatonin in a blank tablet matrix according to the composition of the Stabilimento Chimico Farmaceutico Militare preparations. The recovery

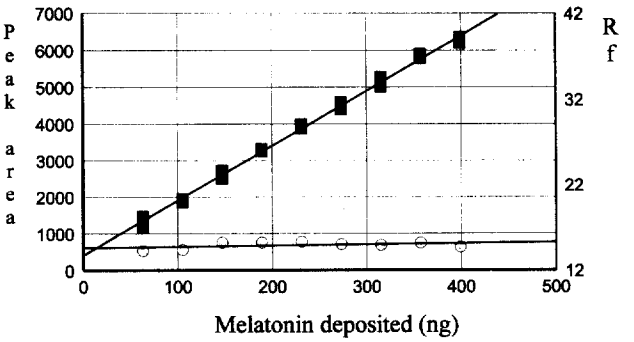

Fig. 3. Peak area (squares) and response factor (circles) vs. amount of melatonin deposited.

obtained was 101.3% ± 1.7 RSD ($n = 6$) and 101.7% ± 1.9 RSD ($n = 6$) for the 10 and 20 mg formulations, respectively.

For the other formulations examined (USA commercial products), however, excipient composition was not available. The analytical results for the investigated preparations are reported in Table 1.

The results reveal that the method reported, applied to the determination of melatonin, is simple, reliable and accurate. Quantitative analysis of pharmaceuticals containing melatonin can be approached using a variety of analytical methods, but this new application of TLC densitometry is cost effective with a short analysis time and a low solvent consumption. Moreover, with a single plate, up to 12 chromatographic runs can be performed simultaneously.

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