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Simultaneous determination of melatonin and pyridoxine in tablets by gas chromatography-mass spectrometry

Luis J. Nùñez-Vergara [a,*], J.A. Squella [a], J.C. Sturm [a], H. Baez, Cristián Camargo [b]

[a] *Laboratory of Bioelectrochemistry, Faculty of Chemical and Pharmaceutical Sciences University of Chile P.O. Box 233, Santiago, Chile*

[b] *Unity of Antidoping and Abuse Drug Control, Faculty of Chemical and Pharmaceutical Sciences University of Chile P.O. Box 233, Santiago, Chile*

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Abstract

A gas chromatographic-mass spectrometric (GC–MS) method for the qualitative and quantitative determination of melatonin plus pyridoxine commercial tablets is described. Melatonin and pyridoxine were simultaneously determined by GC–MS after extraction from ground tablets with methanol and derivatization with *N*-methyl-*N*-*N*-trimethlylsilyltrifluoroacetamide (MSTFA). The mass chromatograms were generated using 232 *m*/*z* ion for melatonin and 280 *m*/*z* ion for pyridoxine, respectively. Splitless injection offers good reproducibility with a standard deviation of 2%. The developed method was applied to analyze the melatonin and pyridoxine content from two different tablet formulations. Also, recovery, detection and quantification limits are reported. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Melatonin; Pyridoxine; GC–MS determination; Tablets; Mass fragmentation

1. Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine) is a hormone synthesized by the pineal parenchymal cells from serotonin by *N*-acetylation and *O*-methylation and secreted by them into the blood and the cerebrospinal fluid. Melatonin synthesis and secretion are increased during the dark period of the day and maintained at low level during daylight hour [1]. Such variation has suggested the possibility of a regulatory function in day/night-dependent physiological processes such as sleep and has led scientists to explore the effects of administered melatonin on the modulation of circadian rhythms [2,3]. From the point of view of therapeutic purposes, melatonin has been successfully used in the treatment of jet-lag effects and other sleep disorders [4,5]. Also, melatonin has been associated with pyridoxine (vitamin B_6), probably due a synergy effective in the therapy of some diseases.

* Corresponding author. Tel.: +56-2-678-2812; fax: +56-2-737-8920.

E-mail address: lnunez@ll.ciq.uchile.cl (L.J. Nùñez-Vergara).

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As more knowledge is gained about melatonin receptors and their roles in physiological processes defined, their importance, as therapeutic targets in pathophysiological states will increase. A great number of papers [6–10] dealing with its quantitative determination has been reported. In some, the application of Gas Chromatography–Mass Spectrometry [9,10] has been used for the determination of melatonin in plasma and cerebrospinal fluid. Such methods resulted in adequate analytical tool, because of its sensitivity and specificity.

The analytical determination of melatonin in pharmaceutical formulations is rather limited to few papers [11,12]. Aboul-Eneim et al. [12], has previously developed a GC–MS method to determine melatonin in tablets. Considering that many pharmaceutical forms combine melatonin and pyridoxine, there is a real necessity to develop analytical procedures to determine both drugs in the same analysis. The simultaneous analysis of melatonin and pyridoxine in tablet formulation by derivative ultraviolet spectroscopy has been recently published [13], but a revision of the literature gives no evidence about GC–MS determination of melatonin and pyridoxine in the same tablet. In the case of pyridoxine, there are many reports about its quantification in pharmaceutical dosage forms, single or in association with other drugs differing of melatonin [14,15].

In the present paper, a novel GC–MS method for the simultaneous determination of melatonin and pyridoxine in tablets is reported. Full mass spectra for drugs, reproducibility test, limit of detection (LOD) and quantification (LOQ) are also reported.

2. Experimental

2.1. Apparatus

2.1.1. GC–MS

Measurements were carried out in a Gas Chromatography/Mass Selective Hewlett Packard 6890/5973 Detector (Palo Alto, California, USA) and an Hewlett Packard 7683 Autosampler. A Hewlett Packard Pentium II Data System, Laser Jet 4000 printer, controlled instrumentation and data handling were also used.

2.1.2. Chromatography column

Hewlett-Packard Ultra-1 column, 25 m × 0.2 mm i.d. × 0.11 film thickness (Little Falls, Wilmington, Delaware, USA).

2.1.3. Chromatographic conditions

Detector temperature, 300 °C; Injector temperature, 250 °C; split ratio, 1/10; pressure, 13 psi; purge flow, 40 ml min^{-1}; purge time, 0.5 ml min^{-1}.

2.1.4. Temperature program

The oven temperature was programmed from 140 to 315 °C (2 min) at 15 °C min^{-1}; run time, 14.67 min. Helium was used as carrier gas with an inlet pressure of 35 kPa. The mass range monitored was 40–450 a.m.u. with a scan rate of 1scan/s and the ionization energy was set at 70 eV.

Peak identification relies upon full spectra comparison of the test samples with certified reference material analyzed within the same batch. There must be a complete agreement with both acceptable chromatography and mass spectrometry.

2.2. Methods

2.2.1. Certified standards

Melatonin (L. S. Row Material Ltd, Tikvah, Israel, Certificate of analysis 1029) and pyridoxine (Takeda Chemical Industries, Ltd, Osaka, Japan, Certificate of analysis HUH 366). These standards were used for preparing the calibration curves, within-day assay precision, and recovery and to determine LOD and LOQ.

2.2.2. Preparation of synthetic samples

For recovery studies ten individual simulated tablets were prepared by weighing 3 mg melatonin and 2 mg pyridoxine certified standards plus excipients (dicalcium phosphate and cellulose), according to manufacturers batch formulas. The procedure to analyze these samples was the same as the individual tablet assay below described.

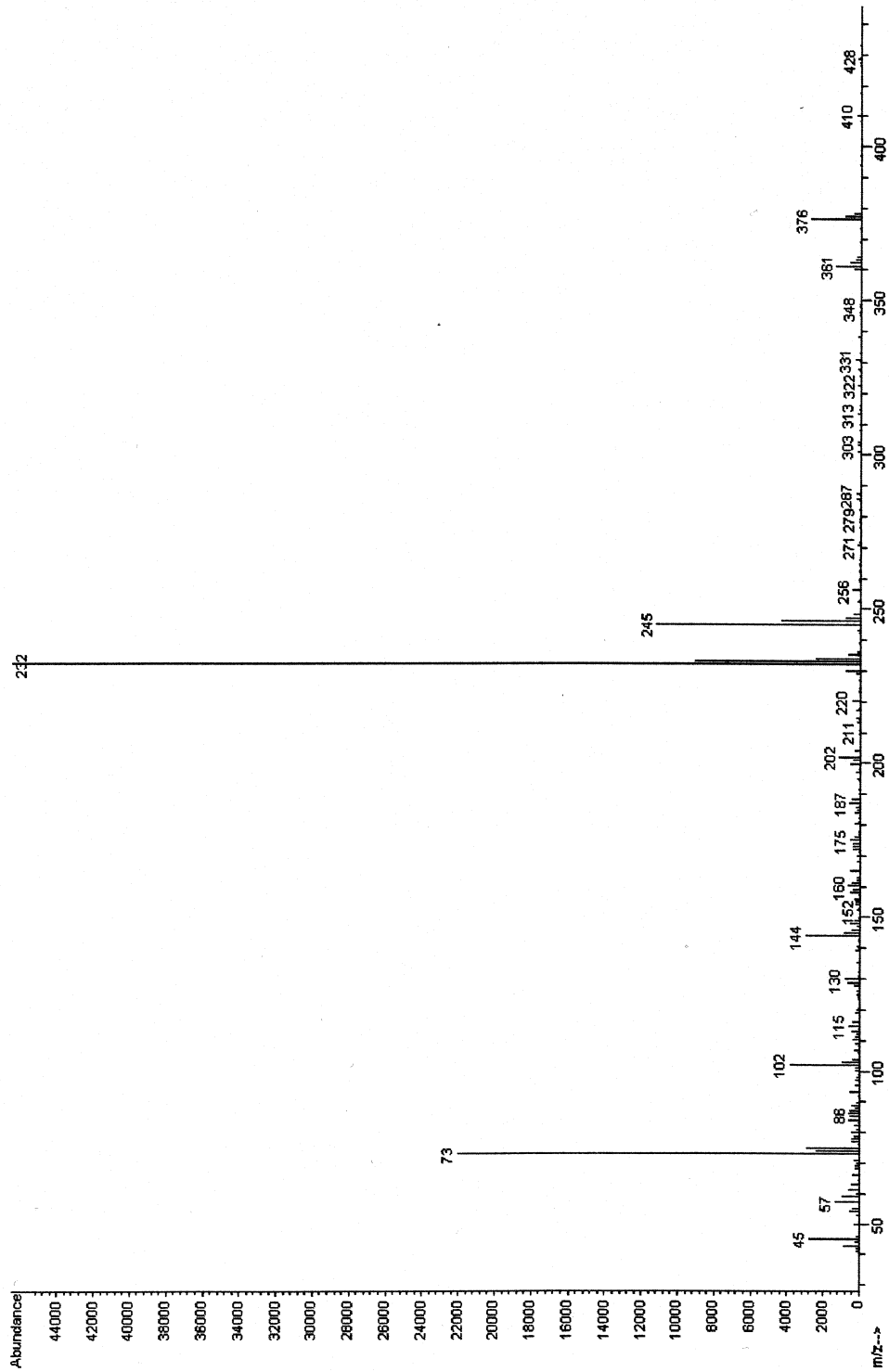

Fig. 1. Mass spectrum of bis-TMS derivative of melatonin.

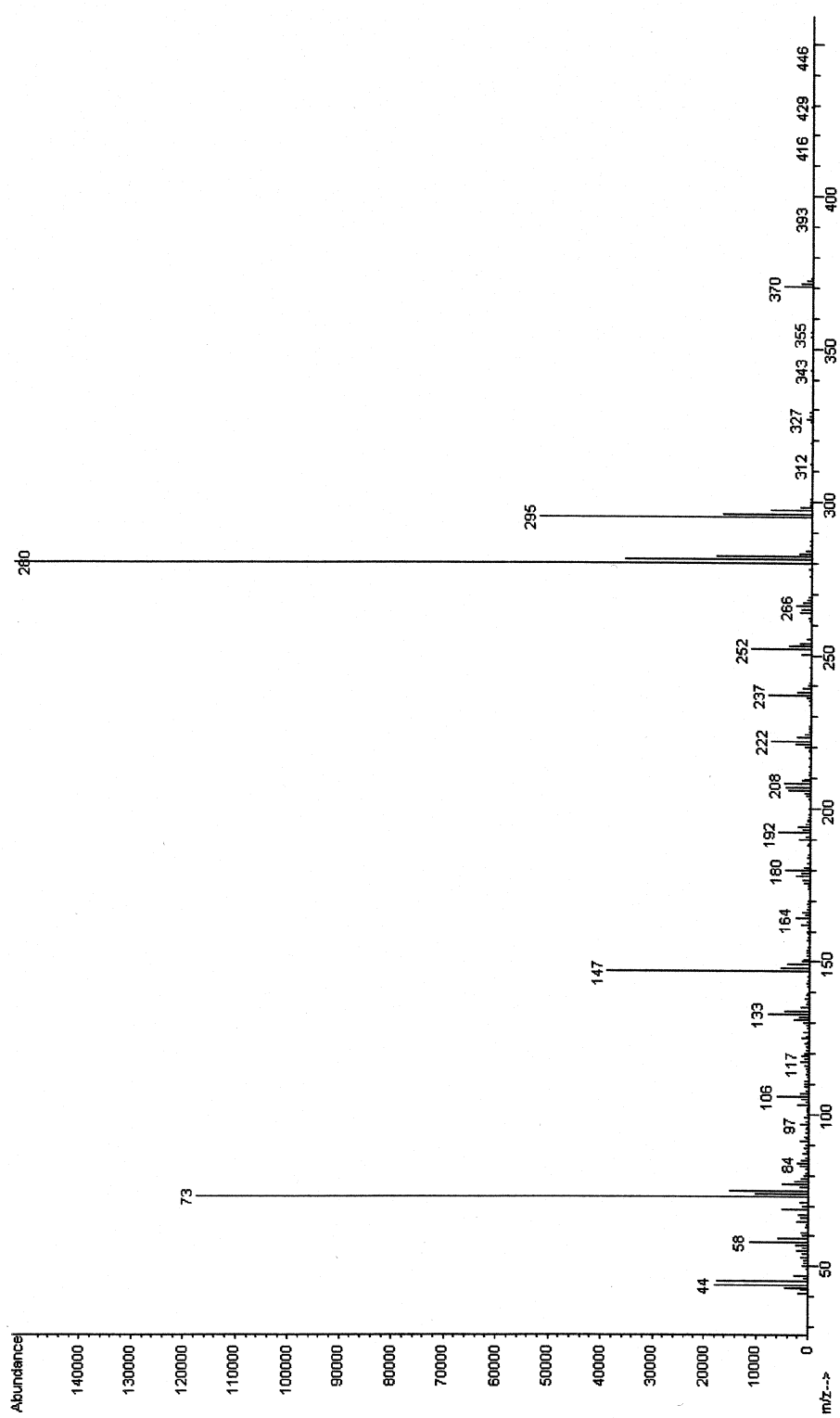

Fig. 2. Mass spectrum of tris-TMS derivative of pyridoxine.

2.2.3. Individual tablet assay

Separately at least 10 tablets of each commercial sample (GNC Laboratories® and Schiff Products®) were taken and finely ground, suspended in 3 ml methanol (Fischer, HPLC grade, New Jersey, USA), transferred into 10 ml glass stoppered tube and sonicated for 20 min to assure the complete dissolution of drugs. Each methanol solution was then filtered through a Whatmann filter paper (N°4) into a 10 ml tube and evaporated to dryness under gentle nitrogen flow, and redissolved with 3 ml of methanol. This stock solution of 1 μg μl^{-1} was diluted to obtain a final working concentration of 10 ng μl^{-1}. Ten microlitre of this solution were evaporated under nitrogen flow, and the residues were dried in vacuum over silica for at least 30 min.

2.2.4. Derivatization

Dry residues of each one either standards or samples were trimethylsilylated with 100 μl of the reagent mixture composed of *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide plus 1% trimethylchlorosilane (MSTFA/TMSCl 100:1) and incubated for 30 min at 75° in a multiblock-heater. Then, splitless injections of 2 μl were analyzed by GC–MS.

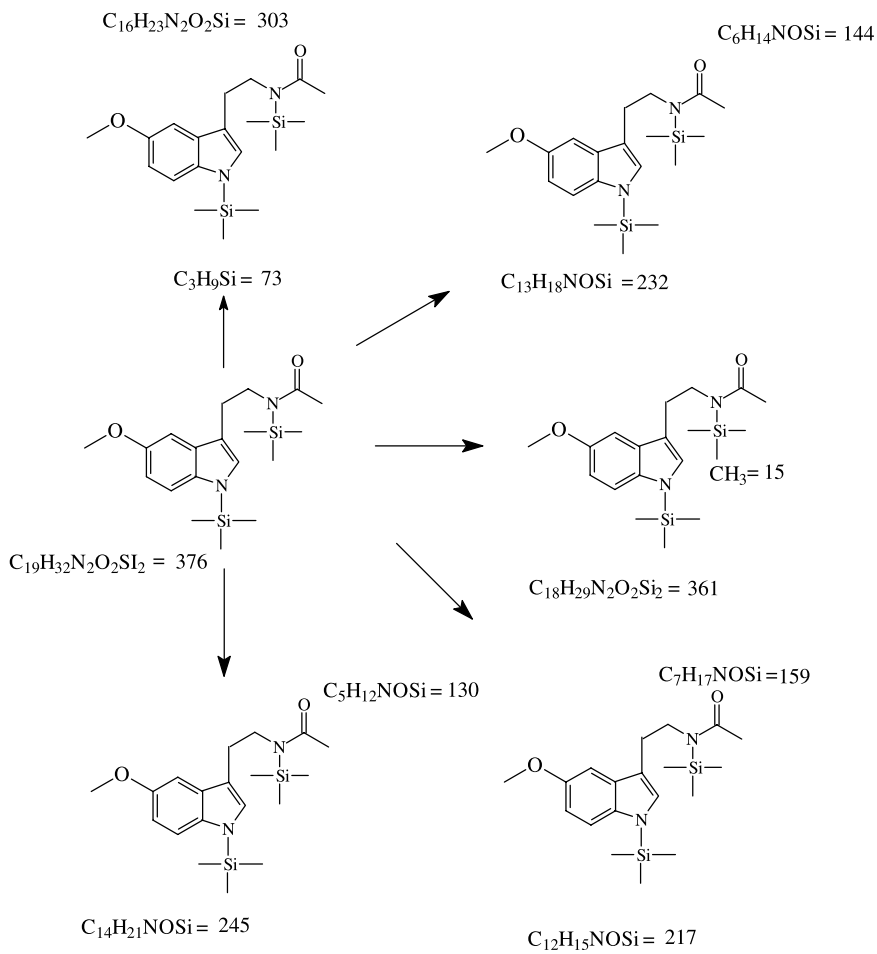

Fig. 3. Scheme of fragmentation proposed for melatonin.

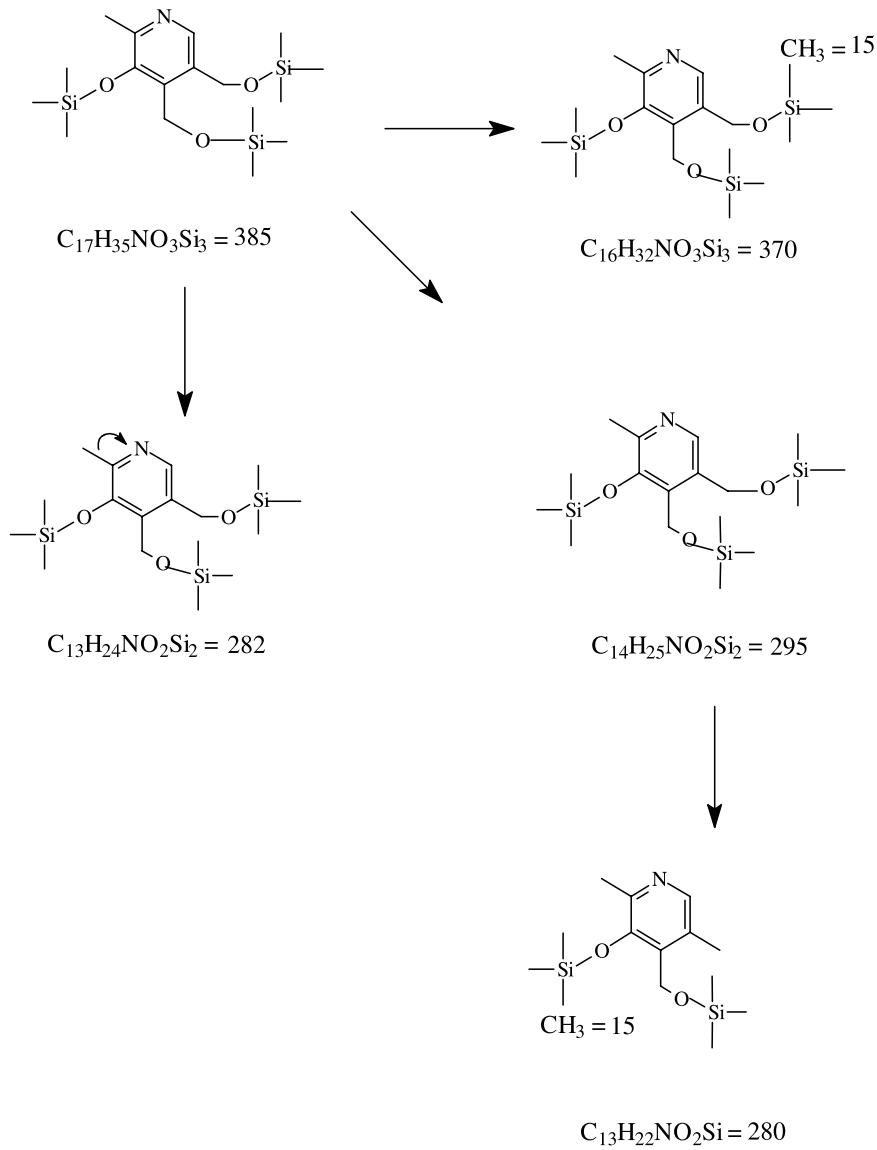

Fig. 4. Scheme of fragmentation proposed for pyridoxine.

3. Results and discussion

The main goal of this work was to study the mass spectra behavior of both melatonin and pyridoxine after derivatization with MSTFA and to explore the applicability of such characteristics to the simultaneous qualitative and quantitative determination of the drugs in different dosage forms. The mass spectrum of mono-TMS derivative of melatonin has only been previously reported [10,12]. In this paper, we have used a derivatization procedure, which gives as final product the corresponding bis-TMS derivative of melatonin, with no indications of the presence of the mono-TMS derivative.

In Fig. 1 and Fig. 2, the mass spectra of bis-TMS-derivative of melatonin and the tri-TMS-derivative of pyridoxine together with the

proposed fragmentation pathways for each one of the drugs are presented.

The measured useful m/z ions for melatonin in the order of significance are the following: m/z $M^+ = 376$, $(M^+ - 15) = 361$, the base peak 232 for quantification, 245, 144. The m/z 73 is a common peak for trimethylsylil derivatives and do not represent an important ion. From these m/z assignments, the quantification and the confirming ions were the following: 232 for quantification, 376, 361, 245 and 144. In Fig. 3, a scheme of fragmentation of melatonin is proposed.

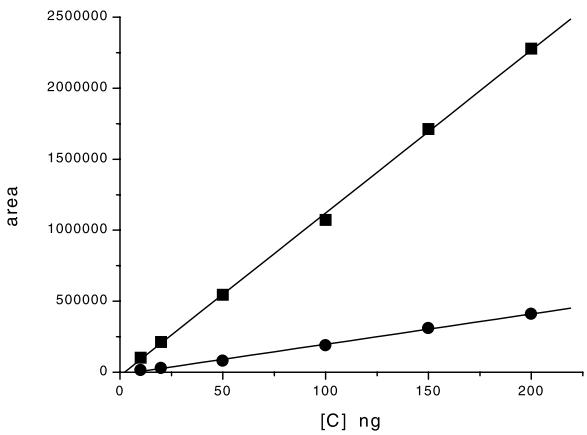

Fig. 5. Calibration curves for bis-TMS derivative of melatonin (■) and for tri-TMS derivative of pyridoxine (●) for levels of concentration varying between 10 and 200 ng/ml.

Table 1
Peak Area reproducibility and their corresponding variation coefficients for melatonin and pyridoxine standards for 6 calibration points

Concentration (ng ml^{-1})	Peak areas	
	Average area[a]	CV, %
Melatonin		
10	14 998	1.39
20	28 027	1.04
50	79 456	1.11
100	189 088	0.70
150	309 843	3.00
200	410 960	0.15
Pyridoxine		
10	102 694	0.99
20	214 263	0.63
50	596 004	2.52
100	1 072 175	1.46
150	1 711 755	1.53
200	2 277 922	0.65

[a] Each average area represents five individual injections of the sample for each concentration.

Table 2
Limits of Detection (LOD) and Quantification (LOQ) for bis-TMS derivative of melatonin and tri-TMS derivative of pyridoxine

Drug	LOD ± SD ng ml^{-1}	LOQ ± SD ng ml^{-1}
Melatonin	5.00 ± 0.08	10.00 ± 0.40
Pyridoxine	2.00 ± 0.06	5.00 ± 0.07

Concerning with mass spectrum of pyridoxine, the m/z values considered useful for its determination were the following: 280 for quantification, 370 and 295 as confirming ions. Fig. 4 shows a scheme for the fragmentation of pyridoxine is also proposed.

Calibration curves of peak areas versus concentration are displayed in Fig. 5 for melatonin and pyridoxine, respectively. Plots exhibited linearity in a wide range (10–200 ng ml^{-1}) for both drugs with the following regression equations for six independent experimental points:

Melatonin:

$$AUC = 2129 \times C \text{ (ng ml}^{-1}\text{)-15923;}$$

with a correlation coefficient of 0.9990

Pyridoxine:

$$AUC = 11464 \times C \text{ (ng ml}^{-1}\text{)-25164;}$$

with a correlation coefficient of 0.9996

Table 1 shows reproducibility data and their corresponding variation coefficients of the proposed method. Results represent five individual injections of samples at six calibration experimental points. As can be seen from the table, data exhibited adequate reproducibility of peak areas at different concentrations ranging from 10 to 200 ng ml^{-1}.

Limit of Detection (LOD) was calculated by measuring standards of decreasing concentrations to establish the lowest concentration that the method can detect with a suitable response in the GC/MS ($S/N = 3$). LOD value for the drugs are shown in Table 2. On the other hand, LOQ, the

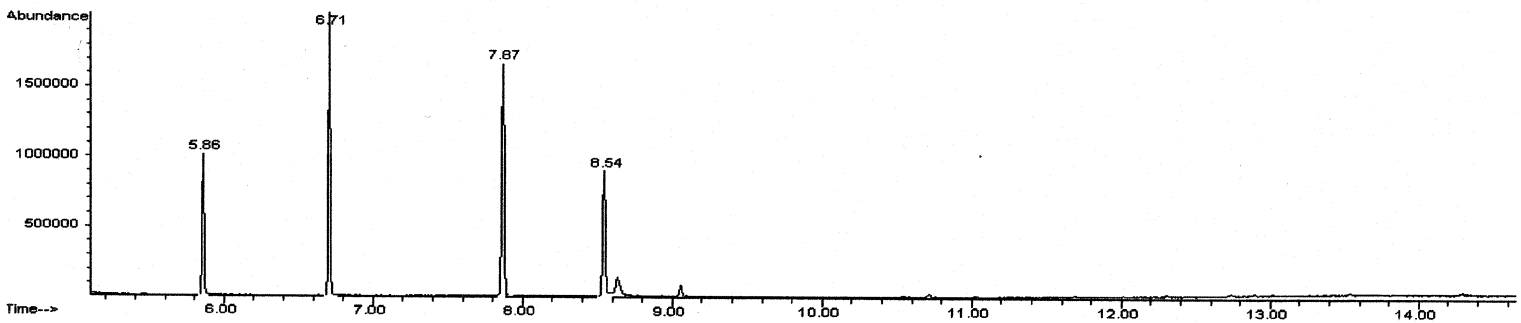

Fig. 6. Total ion chromatogram (TIC) of a commercial tablet of melatonin and pyridoxine (Schiff Products®). Tri-TMS derivative of pyridoxine (5.86 min), bis-TMS derivative of melatonin (8.54 min), C_{16} fatty acid (6.71) and C_{18} fatty acid (7.87 min)

Table 3
Within-day assay precision

Drug	Target concentration	*n*	Mean ng ml^{-1}	SD	CV,%
Melatonin	50	10	50.05	1.09	2.17
Pyridoxine	50	10	50.05	0.89	1.78

Table 4
Melatonin and pyridoxine content from commercial tablets

Pharmaceutical formulations	Drug content (mg drug/tablet)			
	Claimed	Found	SD	CV,%
GNC laboratories				
Melatonin	3.00	3.05	0.06	1.97
Pyridoxine	2.00	1.99	0.05	2.51
Schiff products				
Melatonin	3.00	2.90	0.07	2.41
Pyridoxine	10.00	9.60	0.07	0.73

Drug content were calculated as an average of 10 individual tablet assay.

lowest concentration that the method can quantify at acceptable GC/MS criteria with a S/N of 5. LOQ values are reported in Table 2.

Assay precision within day was estimated by repeatedly analyzing 50 ng ml^{-1} of both melatonin and pyridoxine (certified standards). Results from these experiments show that pyridoxine samples gave the lowest values for both SD and CV, indicating a best precision for this assay (Table 3).

From the recovery studies carried out on synthetic samples containing 3 mg melatonin and 2 mg pyridoxine certified standards, revealed an average recovery of 99.8% (CV, % 0.59) and 99.2% (CV, % 0.62) for melatonin and pyridoxine, respectively. These results indicate that the developed method is precise and accurate.

In relation with pharmaceutical formulations, two different trademark of melatonin + pyridoxine tablets were analyzed by using the developed method. The total ion chromatograms (TIC) were obtained after the individual analysis of 10 tablets containing the amount of drugs indicated in Table 4. Results exhibited an adequate consistency between the claimed and found content, with coefficient of variation lower than 3%, indicating an adequate uniformity content for this type of pharmaceutical dosage forms, according to the analytical criteria of USP 24/NF [16].

The chromatogram corresponding to the tablets (Schiff Products®) is shown in Fig. 6. As can be seen from this Figure, the bis-TMS derivative of melatonin (8.54 min) and the tri-TMS derivative of pyridoxine (5.86 min) are identified, together with long-chain fatty acids C_{16} (6.71 min) and C_{18} (7.87 min), probably added as excipients.

4. Conclusions

From our results it can be concluded that the developed GC/MS method based on the bis-TMS derivative of melatonin and in the tri-TMS derivative of pyridoxine permits the simultaneous determination of the drugs in tablets. The methodology is simple, precise, linear, reproducible, resulting adequate to be applied in pharmaceutical industry to control uniformity of content. The method is also economical because only a minimal amount of solvents and reagents are required.

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