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Short communication

Determination and in-process control of zolpidem synthesis by high-performance liquid chromatography

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Abstract

A high-performance liquid chromatographic assay with diode-array detection has been developed for the in-process control of zolpidem synthesis and for the analysis of the drug and its synthetic intermediates.

The separation uses a 4.6 mm i.d. reversed-phase Kromasil C_{18} (150 mm) column, 5 μm particle size with a gradient elution mode of acetonitrile and 0.02 M NH_4OAc (adjusted to pH 8.0) as the mobile phase (flow rate 1.0 ml min^{-1}).

The analysis is performed in 12 min. The method is simple, rapid and highly specific.

Keywords: Zolpidem; In-process control; HPLC

1. Introduction

Zolpidem (2-(4-methylphenyl)-*N*,*N*-6-trimethyl-imidazo [1,2-*a*]pyridine-3-acetamide), an imidazopyridine derivative, is a nonbenzodiazepine hypnotic agent which combines a rapid onset with a short duration of action. Zolpidem behaves as a sleep inducer without the muscle relaxant and anticonvulsant effects of the benzodiazepines [1].

The analytical techniques published for zolpidem, and eventually some of its metabolites, have mainly involved HPLC [1–12], gas chromatography (GC) [6,7], or even capillary electrophoresis (CE) [13]. To the date, there are no publications concerning the simultaneous analysis of zolpidem and its synthetic intermediates and potential impurities, and it is well known that a well functioning process monitoring system is necessary in the optimization of purification and production processes, and to maintain the conditions at the optimal level required to secure production of high purity zolpidem with maximum yield. The desired yield and purity can only be reached through improved and expanded analytical control, where reversed-phase chromatography (RP-HPLC) has a central role.

Several synthetic routes have been developed for zolpidem synthesis (EP 50563; US 4794185; WO 0008021; US 6281360; EP 1172364; WO 0214306; WO 02090356), and high-performance liquid chromatography (HPLC) analysis is essential for monitoring these production processes, differentiating their corresponding synthetic intermediates.

This paper describes a rapid and reliable gradient HPLC method for the determination of zolpidem and its synthesis intermediates. The utility of the system is demonstrated with the development of applications of industrial significance.

2. Experimental

2.1. Reference compounds

Reference substances zolpidem (2-(4-methylphenyl)-*N*, *N*-6-trimethyl-imidazo[1,2-*a*]pyridine-3-acetamide), 2-am-

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ino-5-methylpyridine, and zolpidem intermediates from Zpd 0 to Zpd VI were supplied by Asturpharma.

2.2. Chemicals

Ammonium acetate for analysis was supplied by Merck (Darmstadt, Germany).

Acetonitrile and methanol, both of gradient grade quality were purchased from Panreac (Barcelona, Spain).

The water used in the mobile phase preparation was first distilled and then deionised in a Milli-Q apparatus (Millipore, Bedford, MA, USA).

2.3. Standard solutions

Standard solutions of zolpidem, its intermediates and the rest of the compounds were prepared at a concentration of 1 mg ml^{-1} by dissolving the appropriate amount of the drug in the mobile phase. Dilutions of the 1 mg ml^{-1} standards were used to make the appropriate working solutions of the drugs.

2.4. Sample preparation

Samples for process control of the different reaction steps were pipetted (25 μl) accurately into 50 ml volumetric flasks and subsequently diluted to the desired volume with mobile phase.

The solutions were sonicated into an ultrasonic bath for 1 min and filtered through a 0.45 μm PTFE syringe filter (Análisis Vínicos, Tomelloso, Spain) and then 20 μl were injected into the HPLC apparatus.

2.5. Chromatography

The chromatographic system consisted of two LC-10ADvp chromatographic pumps, a SIL-10ADvp autoinjector, and a SPD-M10Avp diode-array detector which were all coupled by a programmable system controller SCL-10Avp (Shimadzu, Columbia, MD, USA). Detection took place at 241 nm.

During method development, several prepacked 5 μm particle size columns (150 mm × 4.6 mm i.d.) were employed: Kromasil C_8 and Kromasil C_{18} purchased from Análisis Vínicos (Tomelloso, Spain) and Tracer Excel ODS-A from Teknokroma (Barcelona, Spain).

The column temperature was maintained at 30 °C with the aid of a model MFE-01 temperature controller (Análisis Vínicos, Tomelloso, Spain).

The optimised binary gradient used at a flow rate of 1 ml min^{-1} was as follows: 26% B 0–0.5 min; 55% B 0.5–2.5 min; 55% B 2.5–12.5 min; 26% B 12.5–14.5 min; 26% B 14.5–16 min, where solvent A was 0.02 M ammonium acetate adjusted to pH 8.0 and solvent B was acetonitrile. The final solution was filtered through a Nylon 0.45 μm pore filter (Análisis Vínicos, Tomelloso, Spain) and degassed prior to use.

3. Results and discussion

Among the different synthetic routes of zolpidem, the one outlined in Fig. 1 is the original procedure (EP 50563; Sanofi-Synthélabo). This route seemed to be adequate in terms of yield and simplicity, and in consequence, our efforts have been directed to the optimization of this synthetic procedure, in order to develop an industrial scale method for zolpidem production at Asturpharma's facilities.

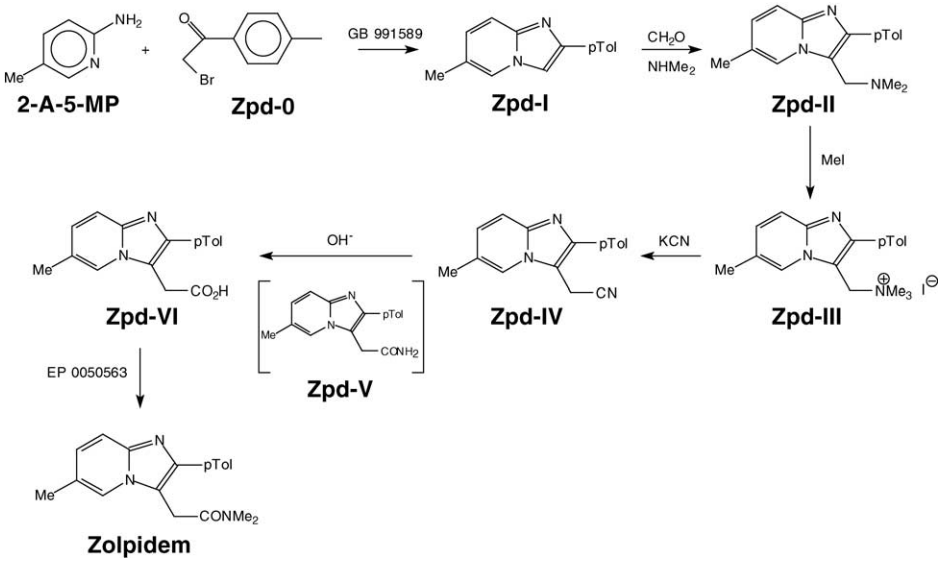

Fig. 1. Synthetic route of zolpidem. Chemical structure for the compounds referred to in the text.

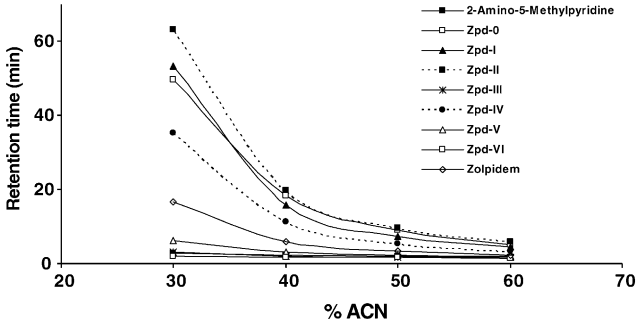

Fig. 2. Influence of acetonitrile content on retention time.

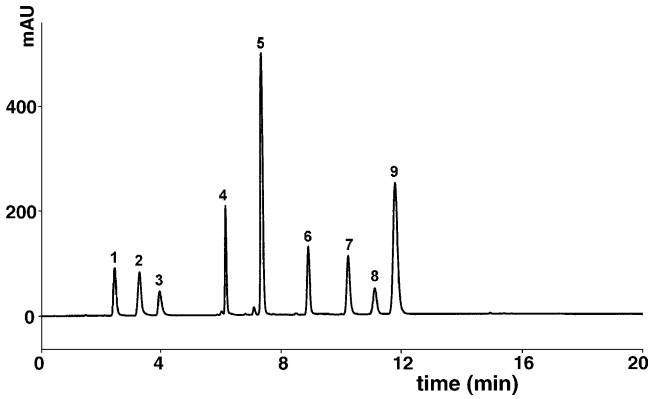

Fig. 3. Chromatogram of a mixture of standards corresponding to zolpidem and its synthetic intermediates. Chromatographic conditions as reported in Section 2.5. Peak identification: (1) Zpd VI; (2) 2-amino-5-methylpiridyne; (3) Zpd III; (4) Zpd V; (5) Zolpidem; (6) Zpd IV; (7) Zpd I; (8) Zpd 0 and (9) Zpd II.

In this context, the study of the influence of the different key variables in the zolpidem production, required the development of an analytical method suitable for identifying and quantifying the different synthetic intermediates and for analyzing the purity of the final product. Based on its properties, HPLC was the analytical tool chosen to carry out our research.

For the chromatographic assay of zolpidem, different chromatographic methods have been proposed [1–12]. In our case, these methods did not prove to be successful, as they were not developed for process control, which was one of the targets in our study, but for zolpidem analysis. During method development, the nature of the stationary phase was evaluated by using Kromasil C_8 and C_{18} chromatographic columns. Kromasil C_{18} gave the best results according to peak symmetry and resolution.

In this study, different pHs and ionic strength were investigated. In order to make the mobile phase compatible with a mass spectrometric detector for the initial identification of compounds, acetic acid, ammonia, and ammonium acetate were employed to set the pH and ionic strength. The best results were obtained between pH 7.0 and 8.0 at 0.02 M ionic strength. In this range, all the compounds are well separated and their peak profile is very good. In consequence, pH 8.0 and 0.02 M ammonium acetate was selected for subsequent experiences.

The type and content of the organic modifier were also checked. Methanol was discarded because it provides poor band spacing. Zpd III/2-amino-5-methylpyridine and Zpd 0/Zpd IV are partially overlapped and moreover, Zpd II is heavily retained.

The chromatographic behaviour of zolpidem and all of its synthetic intermediates with different percentages of acetonitrile is shown in Fig. 2.

As it can be seen in Fig. 2, Zpd III and 2-amino-5-methylpyridine are partially overlapped and moreover, the compounds had large differences in polarity.

Alternatively, different gradient elution programs using acetonitrile as organic modifier were tested. As it can be seen in Fig. 3, which shows the chromatogram of zolpidem and its corresponding synthesis precursors obtained according to these optimised conditions, adequate resolution and good peak symmetry for all the analytes are obtained in a reasonable run time, i.e. 12 min.

3.1. Analytical characteristics

The values presented in Table 1 for the limit of detection (LOD), precision and recoveries show the good performance of the analysis of zolpidem and its intermediates.

The LOD was determined by injecting serial dilutions of a concentrated standard mixture, followed by the preparation of calibration plots, which were extrapolated to a signal-to-

Table 1
Analytical characteristics for the chromatographic determination of zolpidem and its precursors

	Retention time (min)	R^2	LOD (mg l^{-1})	Within-run precision (RSD %)	Recoveries (%)
2-amino-5-methylpyridine	3.4	0.9999	0.1	1.0	98.8–101.1
Zpd0	11.3	0.9997	0.1	1.8	97.1–103.2
Zpd I	10.3	0.9999	0.2	0.4	96.7–100.3
Zpd II	11.8	0.9999	0.1	1.7	99.9–101.9
Zpd III	4.1	0.9998	0.1	1.4	95.9–102.8
Zpd IV	8.9	0.9999	0.1	1.0	98.5–102.0
Zpd V	6.3	0.9999	0.1	1.5	98.4–101.7
Zpd VI	2.6	0.9996	0.3	1.2	96.3–100.8
Zolpidem	7.5	0.9999	0.1	0.5	95.1–100.1

noise ratio (S/N) of three so as to assign the detection limit (according to Winefordner and Long [14]).

Within-run precision was obtained by replicate analyses ($n = 10$) of one mixture of zolpidem and its precursors on the same day. As can be seen, satisfactory results were obtained for all compounds. These range from 0.4–1.8% relative standard deviation (RSD) at mid-calibration range.

The determination of percentage recovery was calculated by comparing the absolute response of the processed (recovered) substances to the absolute response of the external standards. The recovery of zolpidem was determined in triplicate at mid-calibration range. The recoveries were 95.1–103.2%, testifying to the accuracy of the proposed method.

The linearity range was among the quantification limit and at least up to 150 mg l^{-1} for all the compounds.

All the compounds gave a linear correlation coefficient over the studied range greater than 0.999, showing good linear proportionality between concentration and detector response. The linearity of the calibration graphs was checked with the lack-of-fit statistical test. This test evaluates the variance of the residual values [15]. The calculated values were lower than the tabulated ones ($\alpha = 0.01$), linearity thus being demonstrated.

3.2. In-process control

This chromatographic method has been successfully employed to monitorize the influence of different variables (temperature, nature and concentration of the base, etc.) in the synthesis of intermediate Zpd VI.

4. Conclusions

The utility of the method is demonstrated with the development of applications of industrial significance such as the analysis of zolpidem and its synthetic intermediates for in-process control of zolpidem synthesis.

The described method yields good selectivities and precision. In addition, the LOD is in the range of the low mg l^{-1}, and the analysis time is short.

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