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High-performance liquid chromatographic stability-indicating determination of zopiclone in tablets

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Abstract

A high-performance liquid chromatographic method was developed for the determination of zopiclone in pharmaceutical tablets. The ion-pair reversed-phase method utilizes UV absorbance detection and requires about 15 min per analysis. The known potential degradation products of zopiclone are separated, allowing simultaneous determination. The method was validated for reproducibility, linearity, accuracy and limits of detection.

1. Introduction

Zopiclone, 6-(5-chloro-2-pyridyl)-5-(4-methyl-1-piperazinyl)carbonyloxy-7-oxo-6,7-dihydro-5*H*-pyrrolo[3,4-*b*]pyrazine (RP 27267), is a rapid-acting hypnotic drug of the family of cyclopyrrolones [1]. Administered as 7.5-mg tablets under the name Imovane, it improves the length and quality of sleep.

Techniques for the determination of zopiclone include UV and TLC methods [2]. For the development of new formulations and dosage forms, it was necessary to improve the specificity of the assay for the active ingredient and the precision of the determination of small amounts of potential impurities. HPLC methods have been reported [3–8] but are mainly applicable to the determination of zopiclone and metabolites in biological fluids. The difficulty in the development of a quantitative HPLC method is that zopiclone is virtually insoluble in water and ethanol and that potential impurities have great differences in polarity.

This paper describes results obtained with normal- and reversed-phase chromatography and with computer-aided optimization of the ion-pair chromatography of zopiclone and its principal potential impurities. Sample preparation and validation of the method are also included. This method has been successfully employed in product release and stability study testing of zopiclone.

2. Experimental

2.1. Materials, equipment and liquid chromatographic conditions

The chromatographic hardware consisted of a

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Kontron (Saint-Quentin-en-Yvelines, France) Model 420 pump, a Kontron Model 465 autoinjector with variable-volume injection, a Milton-Roy LDC (TSP, Les Ulis, France) Model 3100 UV detector and a Prolabo (Paris, France) Stabitherm column oven regulated at 25°C. Samples in the autoinjector were refrigerated at about 10°C by a Lauda RM6 circulating cryostat (Prolabo). Integration and data storage were carried out on a VG Multichrom system (Fisons Instruments, Chesline, UK). Optimix software (Varian, Les Ulis, France) was used for the simultaneous optimization of chromatographic parameters.

The final choice for the column was a LiChrospher-60 RP Select B cartridge (125 mm × 4.0 mm I.D.) (Merck, Darmstadt, Germany) with a particle diameter of 5 μm. The mobile phase consisted of a buffer with an ion-pair agent–acetonitrile–tetrahydrofuran (81:18:1, v/v/v) flowing at 1.5 ml/min. The injection volume was 20 μl and the wavelength of the UV absorbance detector was 303 nm.

2.2. Reagents and solutions

Monosodium hexane sulphonic acid (Kodak, Rochester, NY, USA), monosodium dehydrogenphosphate (Prolabo), acetonitrile (Distrilab, Caen, France), tetrahydrofuran (THF) (Merck) and water were all of HPLC grade. The buffer was made by adding 3.4 g of monosodium hexane sulphonate ($1.806 \cdot 10^{-2}$ *M*) and 7.0 g of dihydrated monosodium dihydrogenphosphate (0.018 *M*) to 1 l of water. The solution was filtered before use with a 0.45-μm Millipore filter (or equivalent). The mobile phase was made by adding 180 ml of acetonitrile and 10 ml of THF to 810 ml of buffer, mixing thoroughly and degassing with helium. The final pH of the buffer was 4.55, giving an apparent pH of 5.1 for the mobile phase.

2.3. Standard and sample solution preparation

All operations were performed with protection from direct light. For 5.0-mg doses of zopiclone, *ca.* 50.0 mg of zopiclone standard were accurately weighed into a 50-ml volumetric flask, dissolved in 0.1 *M* HCl and diluted to volume. This solution was accurately diluted 1:10 with 0.1 *M* HCl.

For composite sample assay, 20 tablets were accurately weighed and the average tablet mass was calculated. The tablets were triturated to a fine powder. An amount of this powder equivalent to 5 mg of zopiclone was accurately weighed into a 50-ml volumetric flask. About 40 ml of 0.1 *M* HCl were added and treated for 15 min in an ultrasonic bath. The solution was cooled to room temperature and diluted to volume with 0.1 *M* HCl. The resulting solution was filtered through a 1.6-μm glass-fibre filter (Whatman GF/A or equivalent). The standard and test solutions were stable for 24 h when kept in brown glass flasks between 4 and 10°C. For other dosages, *i.e.*, 2.5, 3.75 and 7.5 mg, the dilutions were modified in order to obtain similar final concentrations.

2.4. Procedures

For the uniformity of content test, a 5.0-mg tablet was placed in a 50-ml volumetric flask, about 40 ml of 0.1 *M* HCl were added and the mixture was sonicated until the tablet had completely dissolved. The solution was cooled to room temperature and diluted to volume with 0.1 *M* HCl. The resulting solution was filtered through a 1.6-μm glass-fibre filter (Whatman GF/A or equivalent). A minimum of 10 units were individually analysed.

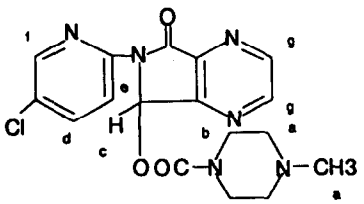
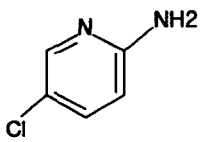
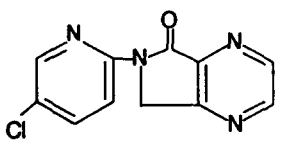
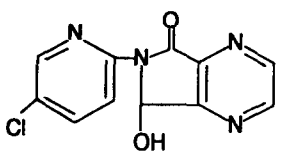
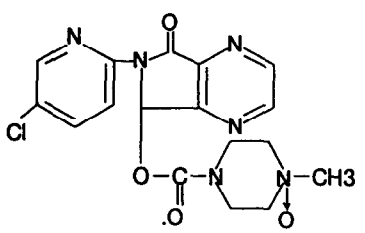
The system suitability was evaluated with a standard solution spiked with 2% of each impurity (see Table 1). The system suitability was determined by injecting 20 μl of spiked standard solution. The resolution between zopiclone and the impurity RP 29753 must be at least 2.0.

The retention time of zopiclone must be between 6.0 and 10.0 min and the column efficiency (N) for zopiclone, measured at the peak half-height, must be >4000 plates/m.

The precision of the system was determined using the relative standard deviation [R.S.D. (%)] of the peak areas for six injections of the standard solution. The R.S.D. must be lower than 2.0%.

Table 1 lists the potential chemical synthesis

Table 1
Capacity factors (k'), plate numbers and relative response factors (RRF) for zopiclone and potential degradation products

Name	Structure	Product	Capacity factor (k')	Column efficiency, N (plates/m)	RRF
Zopiclone (RP 27267)		Active pharmaceutical	12.6	5700	1.00
RP 26695		Potential degradation product	5.5	5100	0.75
RP 48497		Potential degradation product due to bright light	21.4	7900	1.53
RP 29307		Potential chemical synthesis impurity and degradation product	7.9	6500	1.45
RP 29753		Potential degradation product by oxidation	10.1	3700	0.76

impurities and degradation products along with their relative response factors.

Calculations of zopiclone content were made by the external standard method. Impurities can be assayed by the same method. For routine operations relative response factors (RRF, Table 1) are used, because under normal storage conditions no impurity is detected. This allows the

consumption of reference impurities to be minimized. The use of a spiked standard solution with impurities, kept in a cool place, is thus limited for checking the resolution of the chromatographic system.

3. Results and discussion

As zopiclone and its potential impurities are virtually insoluble in aqueous solvents, the choice of the appropriate method was between aqueous and organic dissolution of tablets and between normal and reversed-phase chromatographic modes. In normal-phase chromatography on silica gel, mobile phases of hexane–2-propanol–diethylamine–water (85:15:1.2:0.2) or diisopropyl ether–isooctane–methanol–water–triethylamine (50:35.5:17.5:0.01:0.2) were tried. The presence of a small amount of water allows the control of the activation state of silica [9], and the addition of triethylamine is to maintain zopiclone ($pK_a = 6.7$) and basic impurities in their non-dissociated form. Although the mobile phases used were polar, the potential impurity RP 29753 was not eluted in a reasonable time. Reversed-phase separation on octadecylsilica was tried with an alkaline mobile phase of acetonitrile–water–triethylamine (50:50:0.2), using methanolic solutions of zopiclone and potential impurities. The potential impurities RP 29307 and RP 29753 were not soluble in the mobile phase after injection of 20 μl of 0.1 mg/ml solutions. As the extraction of zopiclone tablets for UV assay was performed satisfactorily with 0.1 *M* HCl, a reversed-phase separation was tried with an acidic mobile phase on a LiChrospher-60 RP Select B column. With acetonitrile–monosodium phosphate buffer (80:20) all peaks were separated on this column. On a classical octadecylsilica column, the peaks were not symmetrical.

The only remaining problem was that the potential impurity RP 26695 was not retained, as was expected for a very polar hydrochloride salt of an amine. However, it was possible to increase its retention by using a hydrophobic counter ion. Monosodium hexanesulphonate was chosen. The influence of the nature of organic solvents was also determined. Acetonitrile or methanol can be used to modify the retention but had little effect on resolution. THF was adopted owing to its positive influence on the peak shape of RP 29307.

With the selected parameters, a multiparametric optimization was performed with Optimix software. This is a PC-compatible version of the CAMPO system [10,11]. The possibility of using non-linear regression permits the prediction of retention times *versus* parameters such as counter-ion concentration, pH and percentage of THF as modifying solvent. After modelling various parameters, the system simulates results for all combinations of conditions. The best operating conditions can be selected according the requirements for resolution, analysis time and pressure. The concentration of monosodium hexanesulphonate was studied in the range 0.005–0.02 *M*, apparent pH in the range 2.0–6.0 and THF concentration from 0 to 4.0 vol.-%. Table 2 gives the results obtained with various THF concentrations.

Other components were 0.02 *M* monosodium hexanesulphonate (pH 4.5)–organic phase (acetonitrile–THF) (80:20, v/vl). The influence of THF content is very variable. It has no effect on

Table 2
Retention times (min) of compounds versus the percentage of THF in 0.02 *M* hexane sulphonic acid (pH 4.5)–acetonitrile (80:20, v/v)

THF (%)	Zopiclone (RP 27267)	RP 29307	RP 26695	RP 29753	RP 48497
0	9.88	6.94	5.22	9.26	18.2
2	12.47	6.95	5.22	9.43	16.7
4	8.79	6.52	5.23	5.06	14.6

the retention of the basic RP 26695. It decreases the retention of RP 48497 as expected. For the other compounds, the retention reaches a maximum with 2% of THF. These behaviours were not predictable and THF concentration of 1% was adopted in order to improve the shape of the peaks.

As expected, the retention of the basic compound RP 26695 is dramatically governed by the formation of an ion pair. However, surprisingly, the retention increased when the pH increased. The same behaviour was observed for zopiclone ($pK_a = 6.7$).

Fig. 1 shows the influence of pH with mixing of two mobile phases at apparent pH 2.0 and 6.0 at various acetonitrile concentrations. After modeling the system with Optimix, an optimum volume composition was simulated. The apparent pH of each mixture was measured. The final composition was chosen because it was obtained naturally with the solution of monosodium phosphate (0.018 *M*), without the need to adjust the pH. After addition of 1% of THF, the final apparent pH was 5.1.

The final composition of the mobile phase giving the best resolution, mainly governed by the pH, was adjusted by varying the organic solvent content. It allows a good baseline separation without the risk of interference from RP 26695, which is eluted with a specific retention. This separation was reproducible with two other batches of analytical column in the same laboratory and two other batches in another laboratory.

The proposed procedure represents a precise, accurate and linear stability-indicating method for the determination of zopiclone and its potential degradation products in pharmaceutical tablets. A typical chromatogram of a spiked standard solution containing each of the components listed in Table 1 is shown in Fig. 2, along with a comparison with a test solution and a placebo. Excipients, (dibasic calcium phosphate, lactose, corn or wheat starch, sodium starch glycolate, magnesium stearate, hydroxypropylcellulose, polyethylene glycol, titanium dioxide) exhibited no interferences with the determination of any of the compounds investigated.

Evaluations of the precision of the method

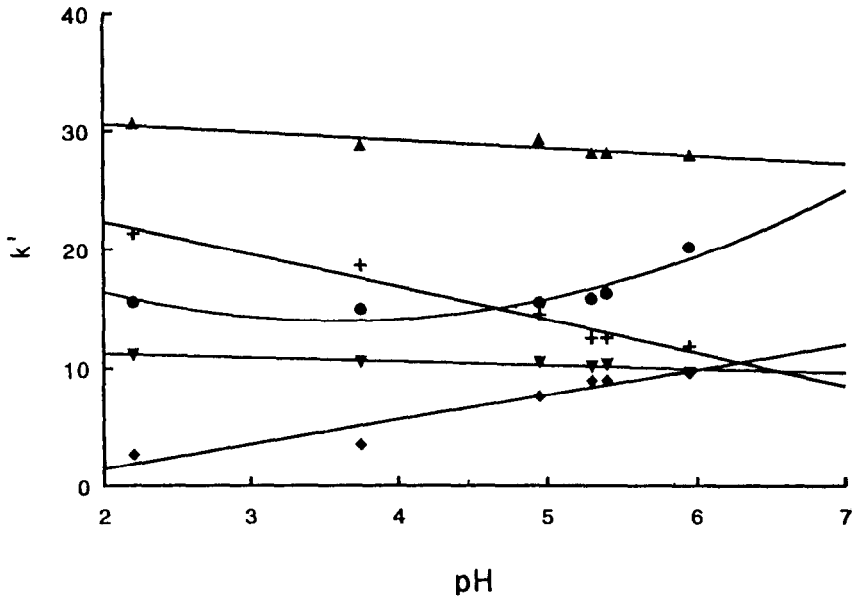


Fig. 1. Influence of pH on capacity factors (k'). Mobile phase, 0.02 *M* monosodium hexanesulphonate in 0.02 *M* monosodium phosphate–acetonitrile (80:20, v/v). ● = zopiclone (RP 27267); ▼ = RP 29307; ◆ = RP 26695; + = RP 29753; ▲ = RP 48497.

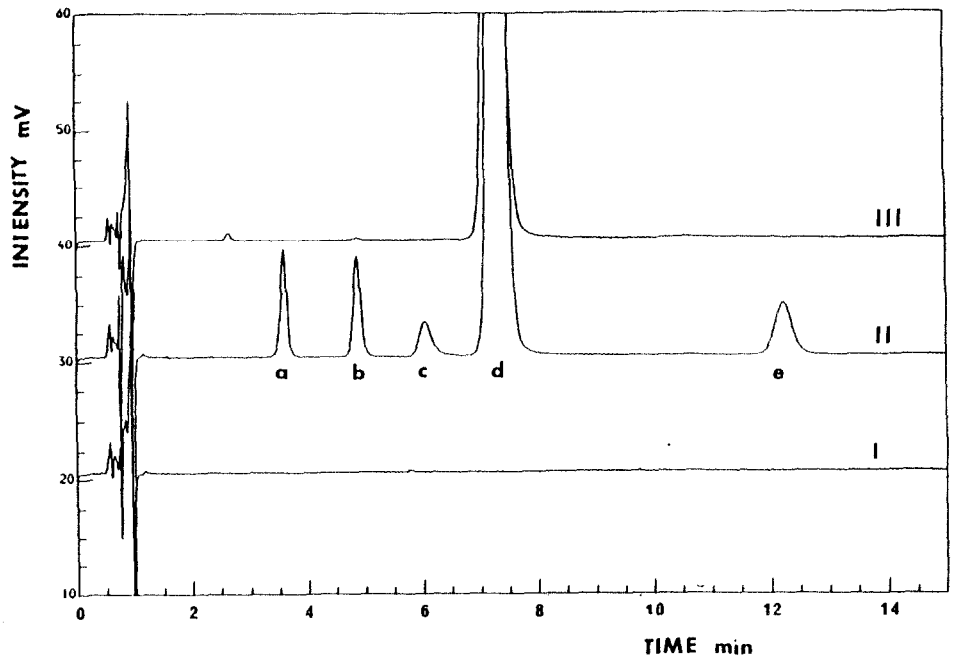


Fig. 2. Typical chromatograms for (I), placebo, (II) spiked standard and (III) test solution. (a) RP 26695; (b) RP 29307; (c) RP 29753; (d) zopiclone (RP 27267); (e) RP 48497.

were made by measuring the standard deviation of ten replicate injections of the same solution and by ten replicate assays of zopiclone. Relative standard deviations of 0.23% and 0.48%, respectively, were found. All these data demonstrate that the method is sufficiently precise to determine the active ingredient and its potential impurities and degradation products.

The recovery of zopiclone was evaluated from 80 to 120% of the labelled tablet amount. The

Table 3
Linearity of response for zopiclone and impurities

Nominal zopiclone content (%)	Concentration (mg/50 ml)	Peak area (μV s)	Peak-area ratio, impurity/zopiclone			
			RP 26695	RP 29307	RP 29753	RP 48497
50	2.555	1 316 117				
75	3.833	1 988 241				
100	5.110	2 649 235				
125	6.388	3 296 203				
150	7.665	3 967 193				
0.5			0.0040	0.0082	0.0044	0.0078
1.0			0.0077	0.0158	0.0080	0.0153
1.5			0.0116	0.0227	0.0120	0.0232
Linear regression[a] ($y = ax + b$)		$y_a = 517446x_a - 803$	$y_b = 0.75x_b + 0.002$	$y_b = 1.45x_b + 0.001$	$y_b = 0.76x_b + 0.004$	$y_b = 1.53x_b + 0.0005$
Correlation coefficient		1.000	0.998	0.989	0.991	0.960

[a] y_a = peak area (μVs); x_a = concentration of zopiclone (mg/50 ml).
y_b = ratio area impurity/area zopiclone; x_b = % impurity.

Table 4
Repeatability of peak areas of potential degradation products for spiked solutions

Impurity (%)	Experimental peak area (μV s)[a]			
	RP 26695	RP 29307	RP 29753	RP 48497
0.2	4410 (7.6%)	8285 (2.2%)	4585 (8.5%)	8878 (5.7%)
0.1	2586 (9.9%)	4365 (9.6%)	2786 (13.8%)	5430 (16%)

[a] Values in parentheses are R.S.D.s ($n = 6$).

recoveries were in the range $100 \pm 2\%$ of the expected amount.

For the potential degradation products, placebo spiked with 0.1 mg (1% of the labelled amount of tablet for 5.0-mg zopiclone tablets) of RP 26695, RP 29307, RP 29753 and RP 48497 gave recoveries of 102.2, 102.9, 99.6 and 99.9%, respectively.

The linearity of the detector response to zopiclone was determined by spiking a placebo mixture of excipients with 50, 75, 100, 125 and 150% of the theoretical tablet amount. Three replicate analyses were performed for each concentration (Table 3).

The linearity of the detector response to each impurity was determined for low concentrations by spiking a standard solution of zopiclone with 0.5, 1 and 1.5% of each individual component. The ratio of the peak area of the impurity to that of zopiclone was measured with six replicate analyses (Table 3). The slope of the regression line gave the relative response factor (RRF) of each impurity (Table 1).

The limit of detection (signal-to-noise ratio = 3) of each impurity is about 0.05% relative to the active ingredient, or 1 ng in 20 μl. The results in Table 4 indicate that 0.1%, relative to the active ingredient, or 2 ng injected in 20 μl, can be determined with an acceptable precision (R.S.D. < 10%) for RP 26695 and RP 29307. For RP 29753 and RP 48497 the limit of determination is about 0.2%.

4. Conclusions

The proposed method provides a rugged, specific procedure for the determination of zopiclone and related compounds in tablets. The sample preparation is simple and allows the accurate determination of all the compounds despite their different chemical structures and solubilities.

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