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Determination of zolpidem hemitartrate by quantitative HPTLC and LC

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Abstract

Two methods are described for the determination of zolpidem hemitartrate in presence of its degradation product. The first method was a TLC-UV densitometric one in which the mobile phase methanol: water (20:80) was used for developing the TLC plates. The R_f of zolpidem hemitartrate was found to be 0.29 ± 0.01 and that of its degradation product was 0.59 ± 0.01. Linearity range was 0.5–4 μg/spot with mean recovery percentage (99.98 ± 0.988)%. The second method was an HPLC method. HPLC was performed on a Bondapack C_{18} column. The mobile phase was composed of a mixture of acetonitrile-0.01 M KH_2PO_4 (40:60). The pH was adjusted to 3.5 ± 0.1. Flow rate was 1.2 ml/min. Calibration graphs were linear in the range of 0.5–5 μg/ml with UV detection at 245 nm. Both methods have been successfully applied to pharmaceutical formulations. The results obtained were statistically compared with those obtained by applying the reported methods.

Keywords: TLC densitometry; HPLC; Zolpidem; Pharmaceutical formulation; Stability indicating assay

1. Introduction

Zolpidem is an imidazopyridine that binds preferentially to one benzodiazepine receptor subtype ω-1 bezodiazepine-1 thought to mediate hypnotic effects. It is an effective hypnotic with only weak anticonvulsant and myorelaxant properties. It is a rapid acting hypnotic agent of relatively short duration of action [1,2].

Many analytical methods have been published for the determination of zolpidem based on HPLC [3,4], GC [5–8], capillary electrophoresis [9] and radioimmunoassay [10].

Zolpidem was determined by HPLC methods together with other drugs and in biological fluids. An additional stability indicating study was done after breaking its amide linkage by acid hydrolysis.

The determination of the cited drug in presence of its acid hydrolysis degradation product by two simple, sensitive and stability indicating chromatographic methods are described. The proposed methods have been applied successfully to tablets containing the drug.

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2. Experimental

2.1. Apparatus

2.1.1. For TLC densitometry

TLC plates used are of the following dimensions: 20 × 20 cm with 0.2 mm thickness silica gel 60 with fluorescent indicator UV_{254} (Macherey-Nagel, Germany). The spots were located using a UV Lamp-short wavelength 254 nm. Scanning was performed using a Shimadzu Dual wavelength-flying CS-9000 spectrodensitometer. Detection was adjusted at λ_{max} 315 nm.

2.1.2. For HPLC method

The chromatographic system consisted of the commercial components: C-R6A chromato PAC Shimadzu, LC-10AD pump, loop injector 20 μl, a Shimadzu SPD-10A UV–Visible detector, operating at 245 nm and sensitivity setting of 0.001. The analytical column was a Bondapack C_{18} 125 Å, 10 μm particle size (4.6 × 250 mm) HPLC cartidge column (Waters). Samples were injected using 25 μl Hamilton® analytical syringe.

2.2. Materials and reagents

Zolpidem hemitartrate was kindly supplied by Amriya for Pharmaceutical Industries, Alexandria, Egypt. Its purity was found to be 100.12%.

Cisapride was used as an internal standard and was supplied by Alpha Chem for Advanced Pharmaceutical Industries Co.

Solvents used as methanol and acetonitrile were purchased from E-Merck, West Germany. Water used was double distilled. Disodium hydrogen phosphate was purchased from El Nasr, while hydrochloric acid used was purchased from E-Merck.

Stilnox tablets were purchased from local pharmacies. Each tablet was labeled to contain 10 mg zolpidem hemitartrate.

Each tablet contains: zolpidem hemitartrate (10.00 mg), lactose (90.40 mg), cellulose microcrystalline (12.10 mg), methylhydroxypropyl cellulose (8.10 mg), sodium carboxymethyl amidon (3.80 mg), magnesium stearate (1.20 mg), titanium dioxide (1.84 mg) and polyethylene glycol (0.56 mg).

2.3. HPLC conditions

The mobile phase used was prepared in the ratio of 40:60 acetonitrile: 0.01% NaH_2PO_4, pH was adjusted to 3.5 ± 0.1. The mobile phase was filtered using 0.45 μm membrane filter and degassed by Ultrasonic vibrations prior to use. The flow rate was adjusted at 1.2 ml/min. Isocratic elution was used. Injection volume was 20 μl. All determinations were performed at 245 nm at room temperature.

2.4. Preparation of acid induced degradation product

0.3 g zolpidem hemitartrate were refluxed with 30 ml 2 M HCl for 2 h. The flask was then cooled, where a white solid was precipitated out of the solution. The precipitate was filtered, washed with few ml-distilled water and dried.

2.5. Standard solutions and calibration graphs

2.5.1. For TLC densitometry

Stock solutions for both zolpidem and its degradation product were prepared by dissolving 0.1 g from each in methanol in two separate volumetric flasks. The final concentration of either solution was 1 mg/ml. As for calibration graph, aliquot portions equivalent to (0.5–4 mg) of zolpidem stock solution were transferred into a series of 5-ml volumetric flasks. The volume was then completed to the mark with methanol. Apply 5 μl of each solution to a TLC plate using 10 μl micro syringe. The plate was then placed in a chromatographic glass tank containing the mobile phase. The plate was developed by ascending chromatography through a distance of 12 cm. The plate was then removed and dried at room temperature. The spots were detected by UV Lamp and scanned by a spectrodensitometer at 315 nm. The area under the peak was then plotted versus the concentration (μg/spot) to obtain the calibration graph.

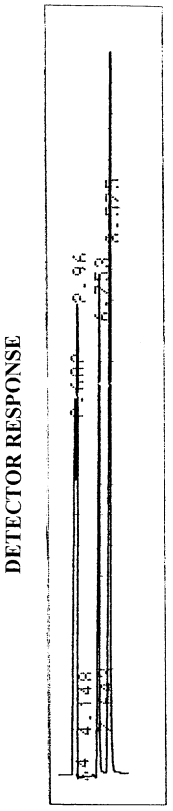

Fig. 1. HPLC chromatogram of 3 μg/ml zolpidem at 6.7 min and internal standard at 8.5 min.

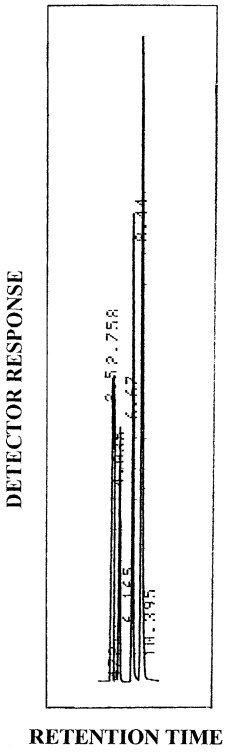

Fig. 2. HPLC chromatogram of zolpidem at 6.67 min, its degradation product at 4.035 min internal standard at 8.44 min.

2.5.2. HPLC

Stock solutions were prepared for both zolpidem and its degradation product, so that the final concentration was 0.1 mg/ml in methanol. These stock solutions were further diluted by methanol to prepare working standard solutions of a concentration 10 μg/ml. Cisapride was used as an internal standard and the concentration of its stock solution was 0.1 mg/ml.

Aliquot portions (0.5–5 ml) of zolpidem working solution and 1.5 ml of internal standard stock solution were transferred into a series of 10-ml volumetric flasks. The flasks were completed to the mark with methanol. The chromatographic procedure was then performed and the mobile phase was prepared of 0.01 M KH_2PO_4: acetonitrile (4:6), the pH was adjusted to 3.5 ± 0.1. It was always filtered using 0.45-μm membrane filters and degassed by Ultrasonic vibrations prior to use. The flow rate used was 1.2 ml/min. All determinations were performed at ambient temperature at 245 nm. The ratios of drug area to internal standard area were obtained, and then plotted versus drug concentrations.

2.6. Assay of zolpidem in tablets

20 Stilnox tablets were finely powdered and weighed; the average tablet weight was determined.

2.6.1. For TLC densitometry

Portion of the powder equivalent to 0.1 g was accurately weighed, then shaken with 100 ml methanol using magnetic stirrer for 30 min. The solution was then filtered through a filter paper

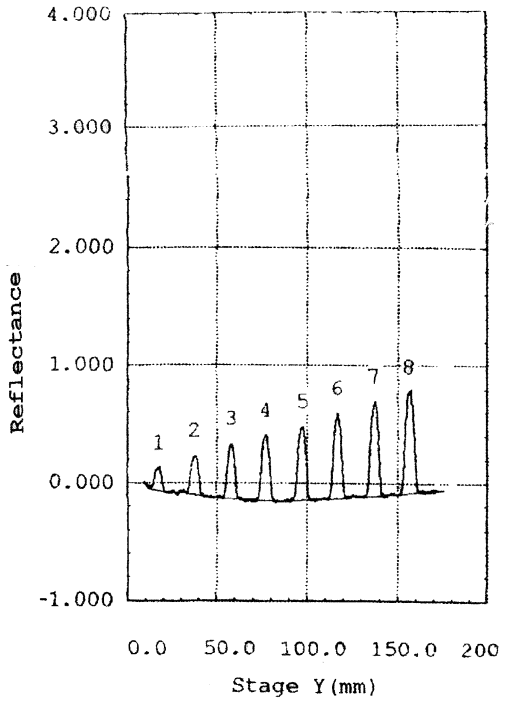

Fig. 3. Scanning profile of TLC chromatogram of 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 μg/spot zolpidem.

followed by filtration through 0.45-μm membrane filters. Samples were completed as in Section 2.5.1, after appropriate dilution was performed.

Table 1
Statistical data for the calibration graphs of zolpidem by TLC densitometric method and HPLC

Parameter	TLC densitometry	HPLC
Linear range	0.5–4 (μg/spot)	0.5–5 (μg/ml)
LOD [14]	0.05 (μg/spot)	0.035 (μg/ml)
LOQ [14]	0.14 (μg/spot)	0.106 (μg/ml)
Accuracy	99.98±0.988	99.96±0.986
Precision	99.50±0.926	100.73±0.570
Intercept (a)	404.04	−0.0033
R.S.D. of intercept	0.168	0.105
Slope (b)	1187.6	0.1766
R.S.D. of slope	0.189	0.232
Correlation coefficient	0.9998	0.9999
Regression equation	A = 1187.6C + 404.04	R = 0.1766C − 0.0033

Where A is the area under the curve (AUC), and R is the ratio of (AUC) of zolpidem: (AUC) of internal standard.

2.6.2. For HPLC method

Powder tablets equivalent to 1 mg was weighed in a 100 ml volumetric flask; volume was completed with methanol. The flask was shaken for 15 min in an Ultrasonic shaker, and then filtered through a filter paper followed by membrane filtration. The procedure adopted in Section 2.5.2 was then used.

2.7. Stability study

2.7.1. For TLC densitometry

The stock solution was mixed with the degradation product in different ratios so that the final spotted concentrations were in the linearity range. Same procedure was followed as in Section 2.5.1 for determination of the drug.

2.7.2. For HPLC method

Working solution of zolpidem was mixed with its degradation product in different ratios within the concentration range of linearity. The procedure adopted in Section 2.5.2 was followed.

3. Results and discussion

Stock solutions of zolpidem and its degradation product in methanol were found to be stable for more than 3 days when kept in refrigerator.

3.1. Optimization of procedures

3.1.1. Optimization of TLC densitometric method

As the structure of the drug contains an amide group and that of the degradation product contains an acid group, polar solvents as methanol and water were suggested to be the components of the mobile phase, and therefore, were tried in different ratios.

Methanol:water (20:80 v/v) gave satisfactory results for the separation of zolpidem and its degradation product, where the R_f of zolpidem was found to be 0.29±0.01 and that of its degradation product was 0.59±0.01. The spots were scanned at 315 and 245 nm; however, the method was found to be more sensitive at 315 nm giving higher peak areas.

Table 2
Determination of authentic zolpidem by TLC densitometry and HPLC

TLC densitometry			HPLC		
Taken (μg/spot)	Found (μg/spot)	Recovery (%)	Taken (μg/ml)	Found (μg/ml)	Recovery (%)
0.5	0.494	98.80	0.5	0.494	98.80
1.0	1.005	100.45	1.0	1.015	101.53
1.5	1.517	101.13	2.0	1.984	99.20
2.0	2.019	100.95	3.0	3.015	101.50
2.5	2.473	98.92	4.0	3.983	99.58
3.0	2.966	98.87	5.0	5.008	100.16
3.5	3.498	99.94			
4.0	4.030	100.75			
Mean ± R.S.D.	99.98 ± 0.988		Mean ± R.S.D.	99.96 ± 0.986	

3.1.2. Optimization of HPLC method

HPLC method described by Debailleul et al. [16] for the determination of zolpidem together with other drugs in plasma and serum was modified. Where C_{18} was used instead of C_8 and the ratio of the components of the mobile phase was changed to resolve the peak of the drug from that of the degradation product.

The mobile phase consisting of KH_2PO_4: acetonitrile (60:40 v/v) of pH 3.5 resulted in a retention time of 6.7 min for zolpidem (Fig. 1). The chromatogram of a mixture of the drug with its degradation product showed an additional peak at 4.1 min Fig. 2. The peak of the degradation product was well resolved from that of the drug.

3.2. Calibration curve of zolpidem

3.2.1. TLC densitometry

Fig. 3 shows the scanning profile of TLC chromatogram of different concentrations of the drug.

A linear correlation was obtained between peak area measured at 315 nm and concentration of the drug. Linearity was found to be in the range of 0.5–4 μg/spot. The regression analysis using the proposed method of least square was made for the slope (b), intercept (a) and correlation coefficient (r) obtained from different concentrations. The results were summarized in Table 1.

3.2.2. HPLC method

Linearity of the method was achieved in a range of 0.5–5 μg/ml. The calibration curve was plotted relating the ratios of drug area to internal standard area and drug concentration. Here also, the regression analysis using least square was made for the slope (b), intercept (a) and correlation coefficient (r). All results were summarized in Table 1.

3.3. Reproducibility, repeatability and precision of the proposed methods

Replicate determination of different concentration levels was carried out and their corresponding concentrations were calculated from the respective regression equations. The mean percentage recoveries and relative standard deviations (R.S.D.) were calculated and were found to be 99.98 ± 0.988 and 99.96 ± 0.986 for TLC-UV densitometric and HPLC methods, respectively, Table 2.

The validity of the proposed methods was assessed by the determination of the drug in its dosage form (Stilnox tablets). Results obtained by applying the standard addition technique are given in Table 3. Mean percentage recoveries of added zolpidem to tablet were found to be 98.47 ± 0.614 for TLC densitometric method and 99.89 ± 0.648 for HPLC method. Furthermore, results of the proposed procedure were statistically compared with those obtained by adopting the Spectroscopic

Table 3
Standard addition technique for determination of zolpidem by TLC densitometry and HPLC

TLC densitometry				HPLC			
Taken	Authentic added (μg/spot)	Authentic found (μg/spot)	Recovery (%)	Taken	Authentic added (μg/spot)	Authentic found (μg/spot)	Recovery (%)
1			99.06	2			98.50
1	1	0.98	98.06	2	1	1.00	100.4
1	1.5	1.49	99.33	2	1.5	1.49	99.33
1	2	1.97	98.50	2	2	2.01	
1	3	2.94	98.00	100.50			
Mean ± R.S.D.	98.59 ± 0.601			2	3	2.98	99.33
				Mean ± R.S.D.	99.61 ± 0.841		

Table 4
Determination of zolpidem hemitartrate in synthetic mixtures with its degradation product by TLC densitometry and HPLC

Degradation product (%)	Recovery (%) of intact zolpidem	
	TLC densitometry	HPLC
10	100.56	98.89
20	98.75	99.50
30	99.29	98.86
40	99.58	99.00
50	101.00	97.60
60	101.88	101.50
80		99.27
Mean ± R.S.D.	100.18 ± 1.175	99.23 ± 1.168

(by dissolving zolpidem in 0.1 M HCl and measuring at 295 nm) company method [13]. Table 5 shows that the calculated t- and F-values are less than the theoretical ones, indicating no significant differences between either of the two proposed methods and the company method.

3.4. Analysis of zolpidem in presence of its degradation product

Zolpidem structure containing an amide can be broken by acid hydrolysis giving two products (Fig. 4) one of which is 6-methyl-2-(4-methylphenyl)-imidazo[1,2-a]pyridine-3-acetic acid and the other is dimethylamine. Our study is confined to

Table 5
Statistical analysis of the results obtained by determination of authentic samples of zolpidem by the proposed TLC densitometry and HPLC as compared with those obtained by company method

Item	TLC densitometry	HPLC	Company method[13]
Mean	99.98	99.96	100.12
S.D.	0.988	0.986	0.620
R.S.D.	0.988	0.986	0.619
N	8	6	8
Variance	0.976	0.972	0.384
t-Value	0.340 (2.145)	0.374 (2.179)	
F-value	2.54 (3.79)	2.53 (4.88)	

Figures in parentheses are the corresponding theoretical t- and F-values ($P = 0.05$) [15].

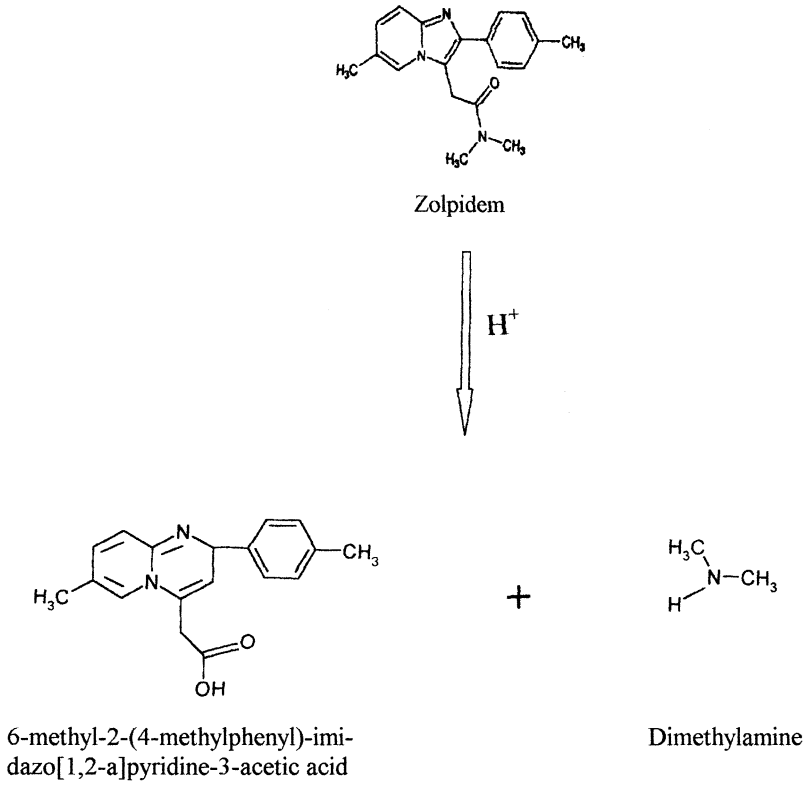

Fig. 4. Suggested mechanism for the acid hydrolysis of zolpidem.

the first degradation product, which is precipitated from the solution after cooling. Dimethylamine has a b.p. 7 °C [12], and therefore, cannot be separated. Zolpidem and the dried precipitate from Section 2.4 were dissolved in methanol and spotted on a TLC plate. Two different spots were observed by the UV Lamp, which proves that the formed precipitate was the degradation product of zolpidem. Also for further confirmation IR was done for both zolpidem and the dried precipitate. IR of the dried precipitate showed the appearance of carboxylic acid group which has a C=O stretching at 1705 per cm (Fig. 5) (C=O stretching of free carboxylic acid is in the range 1725–1700 per cm) [11] and OH stretching at 2621. On the other hand the IR of zolpidem showed C = O stretching of its amide at 1636.5 (C=O stretching of amide is in the range 1670–1630) [11]. This proves that amide linkage was broken.

Table 4 shows the results obtained upon analysis of synthetic mixtures of intact drug and its degradation in different ratios. It is obvious that the proposed TLC densitometric method can be successfully used for selective determination of the intact zolpidem in presence of its degradation product up to 60%. On the other hand, upon application of HPLC method, it was found that the intact drug could be analyzed up to 80% without interference from its degradation product. This proves that the two proposed methods can be used as stability indicating methods.

4. Conclusion

The two proposed methods described in this work were validated as stability indicating methods for the analysis of zolpidem. The proposed

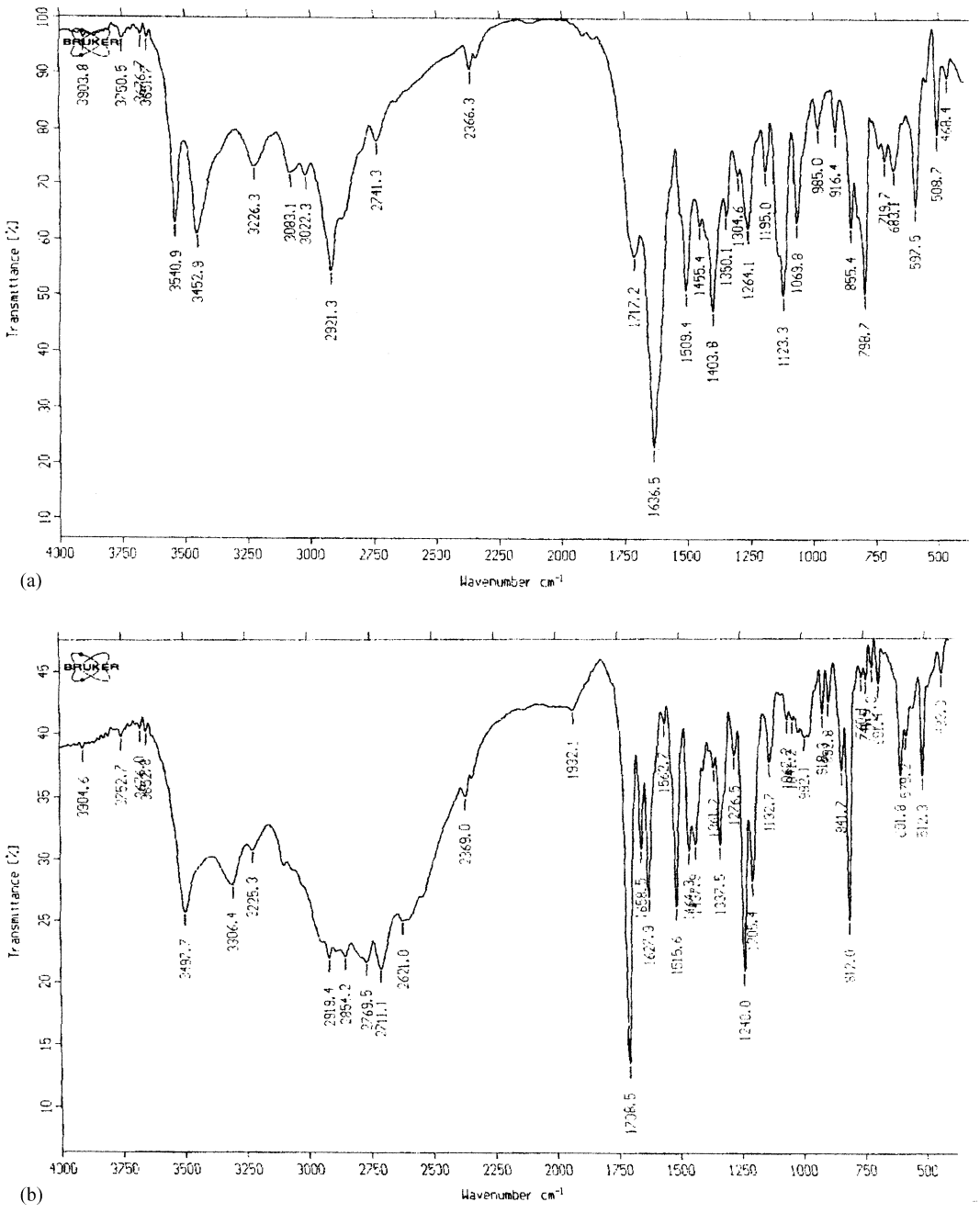

Fig. 5. IR spectrum of (a) zolpidem and (b) its degradation product.

methods are superior over the UV company method determining zolpidem at 295 nm, being more sensitive and a stability indicating methods. Also HPLC method can be applied for the determination of zolpidem in biological fluids having LOD of 0.03 μg/ml.

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