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ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 1277–1282
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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# Quality control Fourier transform infrared determination of diazepam in pharmaceuticals

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Received 10 March 2006; received in revised form 18 October 2006; accepted 19 October 2006
Available online 27 November 2006

**Abstract**

A quality control procedure has been developed for the determination of diazepam in pharmaceuticals using Fourier transform infrared (FTIR) spectroscopy. The method involves the off-line extraction of diazepam with chloroform by sonication and direct determination in the extracts through peak area measurement in the interval between 1672 and 1682 $cm^{-1}$ using a baseline correction defined between 1850 and 1524 $cm^{-1}$. For standardization it was used an external calibration line established from standard solutions of diazepam in chloroform. The method provides a limit of detection of 0.04 mg per tablet ($n = 5$), a relative standard deviation (R.S.D.) of 0.5% for 5 independent measurements of a standard solution at a concentration level of 0.76 mg $g^{-1}$ and a sampling frequency of the whole procedure of 4 $h^{-1}$, being required only 45 s for the measurement step. Results obtained by FTIR agree with those obtained by a reference methodology based on ultraviolet spectrometry and thus the developed procedure offers a good alternative for the determination of diazepam in pharmaceuticals.


*Keywords:* FTIR; Transmittance; Pharmaceuticals; Diazepam; Benzodiazepines; Tablets

## 1. Introduction

Benzodiazepines are depressants used therapeutically to produce sedation, to induce sleep, to relieve anxiety and muscle spasms, and to prevent seizures. In general, benzodiazepines act as hypnotics in high doses, anxiolytics in moderate doses, and sedatives in low doses, being among the most widely prescribed medications [1].

Diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one) is a benzodiazepine compound, which enhances the activity of *gamma*-aminobutyric acid, the most common inhibitory neurotransmitter in the central nervous system. It is used in the treatment of severe anxiety disorders, as a hypnotic in the short-term management of insomnia, as a sedative and premedicant, as an anticonvulsant, and in the management of alcohol withdrawal syndrome [2].

The pharmaceutical regulatory authorities for good manufacturing practices [3] require accurate analysis of finished pharmaceutical products, such as tablets and capsules, to confirm that they contain the required amount of the active ingredient.

There are only few references about the determination of diazepam in pharmaceuticals. Commonly, diazepam has been determined in pharmaceuticals by high performance liquid chromatography (HPLC) [4], micellar liquid chromatography [5] or thin-layer chromatography [6].

Capillary zone electrophoresis [7], voltametry [8] and polarography [9] have been also employed for diazepam determination in tablets.

Ultraviolet spectrophotometric has been used for the diazepam determination based on zero order [10] or first order derivation signals [11]. Additionally, diazepam has been determined in the visible region based on the formation of an ion-association complex with bromocresol green [12] and by flow injection fluorimetry [13]. However, in our knowledge only a precedent exists on the use of vibrational spectroscopy to solve this problem based on the quotient between the absorbance at two wavenumbers in the infrared region [14].

So, the mean purpose of this study has been the development of a fast, accurate and sensitive alternative for the determination of diazepam in pharmaceuticals for quality control of this type of samples.

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doi:10.1016/j.jpba.2006.10.036

## 2. Experimental

### 2.1. Apparatus and reagents

A Nicolet Magna model 750 Fourier Transform Infrared spectrometer (Madison, WI, USA), equipped with a temperature stabilized deuterated triglycine sulphate (DTGS) detector, a KBr Ge coated beam splitter and a globar IR source, was employed for FTIR spectra acquisition, using a Graseby-Specac (Orpington, England) microflow through cell with ZnSe and $CaF_2$ windows and a pathlength of 0.11 mm by averaging 25 scans per spectrum with a nominal resolution of 4 $cm^{-1}$.

The manifold employed permits a fast and reproducible filling and cleaning of the microflow cell for the stopped-flow measurements. It consists of a Gilson P-2 Minipuls peristaltic pump (Villiers-le-bel, France), with Viton (iso-versinic) flexible tubes of 1 mm i.d. and 3 mm o.d., resistant to chlorinated solvents, used to introduce samples and standards into the flow cell.

All the tubes employed to connect the different parts of the manifold are made in Teflon™ and they have 1.57 mm o.d. and 0.8 mm i.d..

Reference procedure measurements were carried out with a Hewlett-Packard Model 8452A diode array spectrophotometer equipped with a 1 cm optical path Hellma quartz cell (Müllheim-Germany).

A J.P. Selecta (Barcelona, Spain) ultrasonic water bath was used to carry out the active ingredient extraction from samples with $CHCl_3$ for FTIR determinations.

Megafuge 1.0 centrifuge (Heraeus Sepatech, Hanau, Germany) was employed to centrifuge samples after extraction in the proposed procedure.

Diazepam standard (100.5%, w/w) was purchased from Guinama (Valencia, Spain) and chloroform (stabilized with amylene), chromatographic gradient grade, was supplied by Scharlau (Barcelona, Spain). Pharmaceuticals analyzed in this study were obtained from the Spanish market with official authorization.

### 2.2. Reference procedure

Twenty tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 4 mg of diazepam, was transferred to a glass-stoppered centrifuge tube, and 40 mL of chloroform were added. The mixture was shaken mechanically for 20 min, centrifuged, and the chloroform extracts transferred to a 100 mL volumetric flask. The extraction was repeated with a 30 mL portion of chloroform, and both extracts combined in the volumetric flask, and finally diluted with chloroform to volume. This solution was transferred to a 250 mL separator, and 40 mL of pH 9.7 borate buffer solution (prepared by mixing 30 mL of 0.1 M sodium hydroxide with 70 mL of a solution made with 12.4 g of boric acid dissolved in 100 mL of 1 M sodium hydroxide and diluted with water to 1000 mL) were added. The mixture was well shaken, and the chloroform layer was passed through a funnel containing a pledget of glass wool layered with 1 g of anhydrous sodium sulfate. Ten milliliters of the filtrate were transferred to a 50 mL volumetric flask, and evaporated under a stream of nitrogen to dryness. The residue was first dissolved using a 1 in 360 solution of sulfuric acid in dehydrated alcohol and then diluted with the same solvent to volume, and mixed. Absorbance of all solutions was determined at 285 nm using the same alcoholic sulfuric acid as the blank in 1 cm cell and an external calibration line of diazepam standards directly prepared in the same acid medium [15].

### 2.3. FTIR proposed procedure

Tablets from each type of pharmaceutical were accurately weighed and powdered. An accurate weight of powder, equivalent to one tablet, was mixed with 7 g of chloroform. The sample was placed inside an ultrasonic water-bath for 5 min, in order to asses the quantitative extraction of the active ingredient. The extract was directly filtrated into a vial using a paper Whatman 42 filter.

FTIR spectra from clear solutions were recorded in the wavenumber range between 4000 and 850 $cm^{-1}$, averaging 25 scans per spectra using a nominal resolution of 4 $cm^{-1}$ and employing a background spectra of the cell filled with the solvent, measured in the same instrumental conditions used for samples.

Peak area values in the interval between 1672 and 1682 $cm^{-1}$, corrected with a two points baseline established between 1850 and 1524 $cm^{-1}$, were employed to quantify diazepam in samples using an external calibration line obtained with six standard solutions of diazepam dissolved in chloroform, covering a concentration range from 0.16 to 2.14 mg $g^{-1}$, measured in the same conditions than samples.

## 3. Results and discussion

### 3.1. FTIR spectra of diazepam

Spectra of both, samples and standards were obtained in KBr disks and in solution with chloroform.

As depicted in Fig. 1, the FTIR spectrum of a diazepam standard diluted in potassium bromide disks exhibits numerous well defined bands, thus indicating that direct sample measurements, such as solid attenuated total reflectance (ATR) [16] or diffuse reflectance (DRIFT) [17], could be an alternative for a fast sample analysis, but using also multivariate calibration techniques. However, the presence of high quantities of lactose and cellulose, used as excipients in the pharmaceuticals under study, prevents the direct determination of diazepam using univariate calibration models and, because of that, a previous separation of the active principle from the samples is required.

In this sense, Fig. 2 shows the FTIR spectra of chloroform solutions of a diazepam standard and the extracts obtained from samples, all of them obtained using a background established with the cell filled with chloroform. As can be seen, spectra of samples and standard are very similar and present several coincident vibrational bands suitable to be used for direct determination of diazepam in commercially available tablets.

As it can be seen in Fig. 2, the most intense band present for a chloroformed diazepam standard and extract is centered at

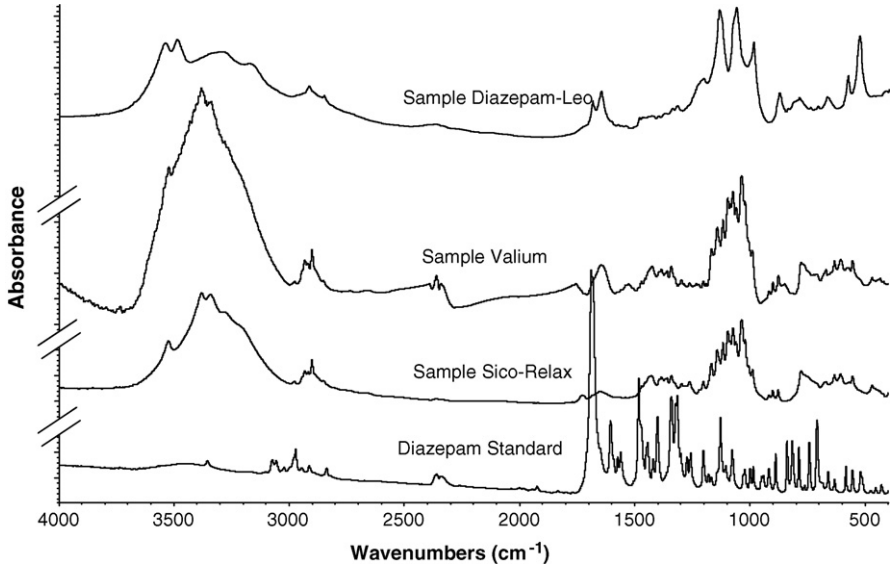


Fig. 1. FTIR spectra, in KBr disks, of a diazepam standard and three pharmaceuticals used in this study. Note: spectra were shift on the absorbance axis to clearly show their bands. Instrumental conditions: 25 scans, 4 $cm^{-1}$ nominal resolution.

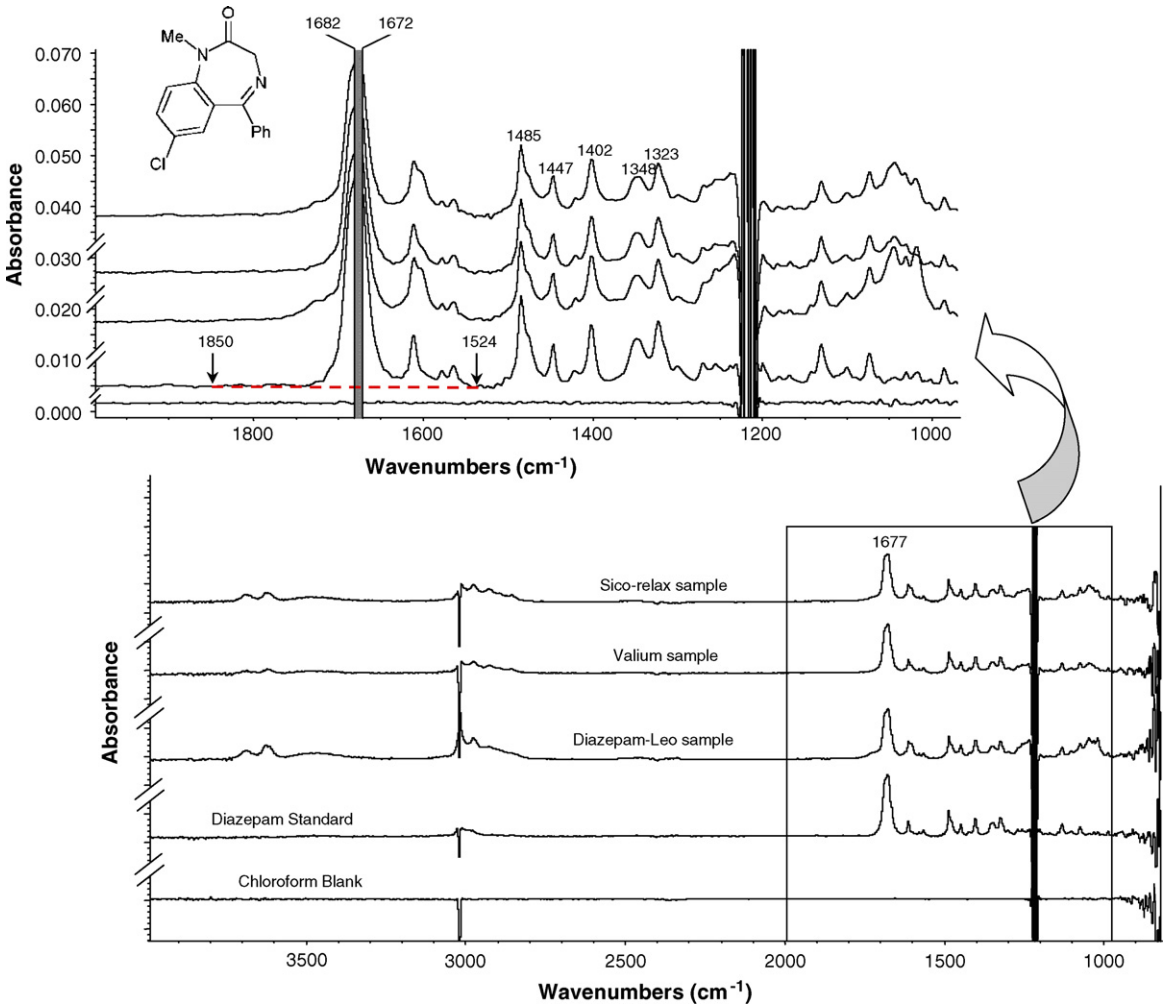


Fig. 2. FTIR spectra of a diazepam standard in chloroform at a concentration level of 1 mg $g^{-1}$ and extracts of each one of the three pharmaceuticals assayed. Instrumental conditions: 25 scans averaged per spectra, 4 $cm^{-1}$ nominal resolution using a background of the cell filled with chloroform. Note: spectra were shift on the absorbance axis to clearly show their bands.

1676 $cm^{-1}$ and it can be related to the presence of a carbonyl group (C=O). Other less intense bands that are located at 1485, 1447, 1402, 1348 and 1323 $cm^{-1}$ could be associated to the in-plane C–H bending vibrations of the benzene ring that interacts (sometimes strongly) with various ring CC vibrations [18].

### 3.2. Effect of instrumental and experimental conditions

The effects of the number of cumulated scans and the spectral resolution employed for data acquisition were evaluated in order to improve the measurement conditions. With this purpose, a monoparametric study was performed. In this sense the number of cumulated scans per spectrum was modified from 5 to 75, working with a fixed spectral resolution of 4 $cm^{-1}$, and also the spectral resolution varied from 2 to 8 $cm^{-1}$ averaging 25 scans.

As can be seen in Fig. 3A, working with peak area values in the interval between 1672 and 1682 $cm^{-1}$ corrected with a two points baseline established between 1850 and 1524 $cm^{-1}$, when 50 and 25 scans were cumulated, the most intense and precise results were achieved, but the time required for spectrum collection was two times higher for 50 scans than that required for 25. Thus, in order to ensure a compromise between time-consumption for developing the whole procedure, sensitivity and precision, 25 scans were selected, that involves a measurement time of 45 s per spectrum.

On the other hand, it can be observed in Fig. 3B that the best signal to noise ratio was found for a 4 $cm^{-1}$ nominal resolution.

To carry out the extraction of diazepam from tablets, two extraction modes, mechanical and ultrasonic, were tested using different times from 1 to 15 min. Fig. 4 shows the effect of both shaking modes on the diazepam extraction yield and as it can be seen sonication with $CHCl_3$ for 5 min are enough to achieve diazepam quantitative extraction, being required 10 min of mechanical shaking to obtain the same results.

Additionally, it can be appreciate a slight decrease of the extraction percentage for an over dimensioned sonication time. As it has been reported previously, in other cases [19] this fact seems to be related with damage on the active principle molecules due to the generation of free radicals.

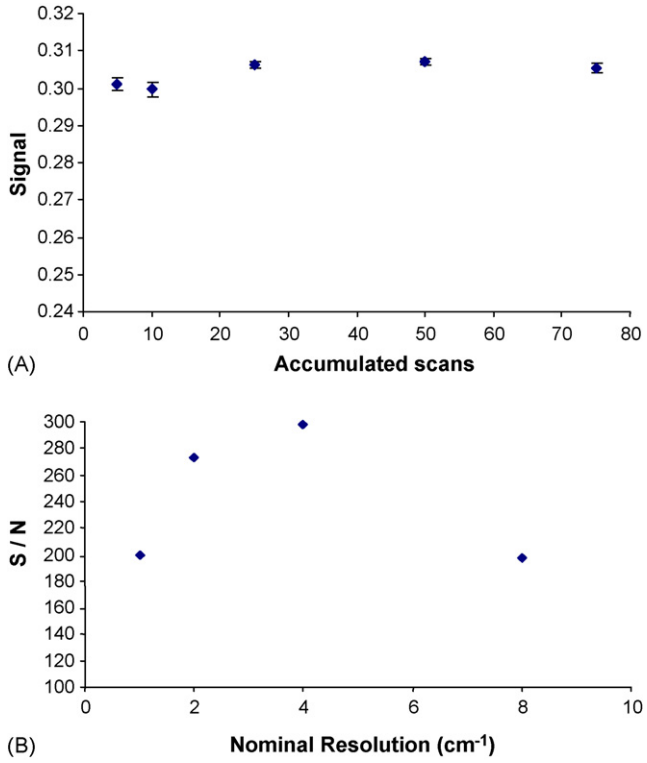


Fig. 3. Effects of the averaged scans per spectrum (A) and the nominal resolution (B) employed to carry out the instrumental measurements. The study was carried out using a standard with 1 mg $g^{-1}$ concentration and the measurements were obtained working with area values for the interval between 1672 and 1682 $cm^{-1}$ with a baseline correction between 1850 and 1542 $cm^{-1}$.

### 3.3. Selection of bands for FTIR determination of diazepam

In order to choose the best analytical performance of the FTIR determination of diazepam in pharmaceuticals, different bands and baseline correction criteria were evaluated, as can be seen in Table 1. In every case, it was also considered the use of peak height and peak area absorbance measurement modes.

In terms of sensitivity, taking into consideration the calibration slope values, it is clear that peak area measurements provide at least one order magnitude better sensitivity than peak

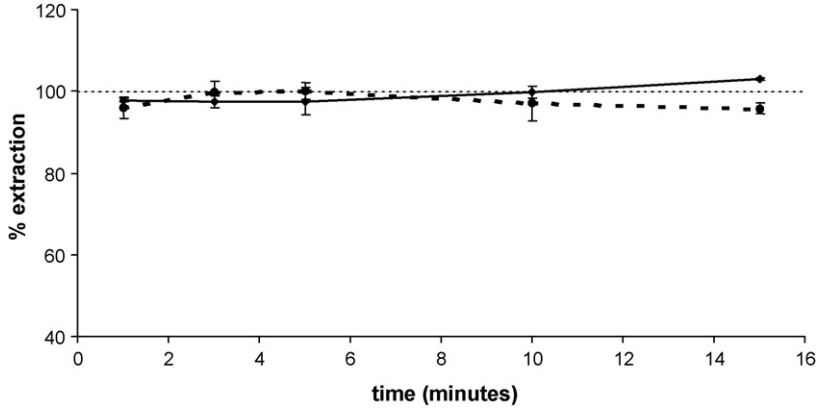


Fig. 4. Effect of increasing extraction times to recover diazepam from pharmaceuticals using both, mechanical (—) and ultrasonic (- - -) shake modes. The extraction percentage was calculated using the concentration of diazepam in the sample found by the reference procedure. Values indicated correspond to the average of three independent determinations ± the standard deviation.

Table 1
Analytical features of the FTIR determination of diazepam in pharmaceuticals using peak height and peak area criteria with different baseline corrections

| Peak height ($cm^{-1}$) | Baseline correction ($cm^{-1}$) | External calibration line[a] $y=(a\pm CL_a)+(b\pm CL_b)$ [Diaz] | $r^2$[b] | % R.S.D.[c] | LOD[d] | LOQ[e] |
|---|---|---|---|---|---|---|
| 1677 | 1850–1524 | $y=(0.0009\pm0.0004)+(0.04922\pm0.0004)$ [Diaz] | 0.9997 | 0.6 | 0.04 | 0.13 |
| | 1850–1434 | $y=(0.0006\pm0.0004)+(0.0485\pm0.0004)$ [Diaz] | 0.9997 | 0.2 | 0.04 | 0.12 |
| 1485 | 1524–1434 | $y=(0.00038\pm0.00016)+(0.02007\pm0.00015)$ [Diaz] | 0.9997 | 0.9 | 0.16 | 0.5 |
| | 1524–1384 | $y=(0.0005\pm0.0002)+(0.02027\pm0.00019)$ [Diaz] | 0.9996 | 0.9 | 0.2 | 0.7 |
| 1447 | 1524–1434 | $y=(0.00006\pm0.00011)+(0.00835\pm0.00010)$ [Diaz] | 0.9993 | 2 | 0.4 | 1.3 |
| 1402 | 1434–1283 | $y=(0.00017\pm0.00009)+(0.01238\pm0.00009)$ [Diaz] | 0.9998 | 2 | 0.14 | 0.5 |
| 1348 | 1385–1283 | $y=(0.00028\pm0.00012)+(0.00891\pm0.00011)$ [Diaz] | 0.9993 | 1.3 | 0.3 | 1.1 |
| 1323 | 1385–1283 | $y=(0.00025\pm0.00012)+(0.01282\pm0.00011)$ [Diaz] | 0.9997 | 0.6 | 0.18 | 0.6 |
| **Peak area ($cm^{-1}$)** | **Baseline correction ($cm^{-1}$)** | **External calibration line[a] $y=(a\pm CL_a)+(b\pm CL_b)$ [Diaz]** | **$r^2$[b]** | **% R.S.D.[c]** | **LOD[d]** | **LOQ[e]** |
| 1682–1672 | 1850–1524 | $y=(0.005\pm0.004)+(0.457\pm0.003)$ [Diaz] | 0.9998 | 0.5 | 0.04 | 0.14 |
| | 1850–1434 | $y=(0.002\pm0.004)+(0.451\pm0.003)$ [Diaz] | 0.9997 | 0.3 | 0.04 | 0.12 |
| 1700–1654 | 1850–1524 | $y=(0.015\pm0.012)+(1.415\pm0.011)$ [Diaz] | 0.9997 | 0.9 | 0.08 | 0.3 |
| | 1850–1434 | $y=(0.001\pm0.012)+(1.383\pm0.011)$ [Diaz] | 0.9997 | 0.5 | 0.05 | 0.18 |
| 1488–1479 | 1524–1434 | $y=(0.0027\pm0.0018)+(0.1552\pm0.0017)$ [Diaz] | 0.9994 | 1.1 | 0.2 | 0.7 |
| | 1524–1384 | $y=(0.004\pm0.002)+(0.157\pm0.002)$ [Diaz] | 0.9992 | 1.2 | 0.3 | 0.9 |
| 1453–1441 | 1524–1434 | $y=(-0.0005\pm0.0014)+(0.0685\pm0.0013)$ [Diaz] | 0.998 | 3 | 0.5 | 1.5 |
| 1407–1396 | 1434–1283 | $y=(-0.0005\pm0.0012)+(0.1051\pm0.0011)$ [Diaz] | 0.9995 | 3 | 0.13 | 0.4 |
| 1333–1306 | 1385–1283 | $y=(0.003\pm0.002)+(0.199\pm0.002)$ [Diaz] | 0.9995 | 1.1 | 0.2 | 0.7 |

[a] Calibration curve where $(a\pm CL_a)$ and $(b\pm CL_b)$ correspond to the intercep and the slope, respectively ± their corresponding confidence limits (for a 95% confidence level), being [Diaz] the concentration of diazepam expressed in mg $g^{-1}$.
[b] Correlation coefficient.
[c] % relative standard deviation (for five independent measurements) calculated for a standard of 1 mg $g^{-1}$ concentration.
[d] Limit of detection expressed in mg per tablet and calculated using 3 s criteria, where s corresponds to the standard deviation of five measurements of the blank solution.
[e] Limit of quantification expressed in mg per tablet and calculated using 10 s criteria, where s corresponds to the standard deviation of five measurements of the blank solution.

height values, but limit of detection data are comparables for both measurement criteria applied to a same band. Moreover, data in Table 1 also report the limit of detection (LOD) and the limit of quantification (LOQ) values and the relative standard deviation (R.S.D. %) found on using the different criteria. As it can be seen, several studied conditions provided appropriate characteristics for pharmaceutical analysis.

The band at 1677 $cm^{-1}$ provides the best precision, sensitivity and limit of detection/quantification values. However, as it can be seen in Fig. 2 for sample spectra there is a little shoulder near the selected band and it does not present a good symmetry, probably due a possible spectral interference from other sample ingredients. Because of that we have considered to work with a peak area interval centered in the maximum of the band, between 1682 and 1672 $cm^{-1}$ and using a baseline correction defined between 1850 and 1524 $cm^{-1}$ or, alternatively, a baseline fixed between 1850 and 1434 $cm^{-1}$.

## 4. Analysis of commercial available pharmaceuticals

In order to validate the developed FTIR procedure, three pharmaceuticals containing diazepam were analyzed by both, the FTIR developed procedure and the UV reference method, and results found are summarized in Table 2.

Table 2
Determination of diazepam in pharmaceuticals by UV and FTIR procedures

| Conditions for FTIR determination | | (mg per tablet)[a] | | Relative accuracy error (%)[b] | $t_{exp}$[c] | % recovery[d] |
|---|---|---|---|---|---|---|
| Peak area ($cm^{-1}$) | Baseline correction ($cm^{-1}$) | FTIR method | UV method | | | |
| 1682–1672 | 1850–1524 | 5.02 ± 0.05 | 4.98 ± 0.05 | 0.8 | 1.403 | 98.2 ± 0.9 |
| | | 4.95 ± 0.05 | 4.96 ± 0.04 | −0.2 | 0.442 | 101.6 ± 1.4 |
| | | 4.929 ± 0.011 | 4.91 ± 0.04 | 0.4 | 1.347 | 104.1 ± 1.2 |
| | 1850–1434 | 5.07 ± 0.05 | 4.98 ± 0.05 | 1.8 | 3.157 | 98.7 ± 0.9 |
| | | 4.93 ± 0.03 | 4.96 ± 0.04 | −0.6 | 1.697 | 102.3 ± 1.0 |
| | | 5.077 ± 0.019 | 4.91 ± 0.04 | 3 | 10.667 | 104.3 ± 0.7 |

[a] Concentration values are the average of three independent triplicate analyses ± their standard deviation. The three pharmaceuticals analyzed correspond, from the top to the bottom, to Diazepam-Leo, Valium and Sico-relax.
[b] % error calculated as ([FTIR] − [UV] × 100)/[UV], where [FTIR] and [UV] belong to the concentrations found using both assayed procedures, for each one of the samples analyzed.
[c] $t_{tab}$ = 2.120 with a probability level of 95% and 16 freedom degrees (3 independent analysis measured by triplicate for both methodologies).
[d] Mean % recovery ($n$ = 3) of diazepam added to the three pharmaceutical samples ± the corresponding standard deviation.

It was confirmed that using a baseline correction established between 1850 and 1524 $cm^{-1}$, data found by FTIR agree better than those obtained by UV than in the case of using 1850–1434 $cm^{-1}$ correction.

Additional experiments made on spiking real samples with diazepam amounts from 2.5 to 8.5 mg provided recovery percentage from 98.2 to 104.1% in the selected conditions.

On the other hand, the statistical comparison of paired results, also summarized in Table 2, provided $t_{exp}$ values that are lower than 2.120, the theoretical $t$ value for a confidence level of 95% and 16 freedom degrees, thus indicating that results of both procedures are statistically comparables on using the selected conditions.

## 5. Conclusions

The proposed transmittance FTIR procedure offers an appropriate alternative to the reference UV method for the determination of diazepam in tablets. Accurate and precise results are obtained using a simple external univariate calibration. This method provides an adequate sensitivity and precision, with a limit of quantification of 0.14 mg per tablet, with a repeatability of 0.5%, only requiring 7 g of $CHCl_3$ per sample instead the 100 mL $CHCl_3$ plus 50 mL ethanol consumed by the UV reference method. The sampling throughput was 4 $h^{-1}$ in front of 0.5 $h^{-1}$ for the UV method and the measurement step requires only 45 s per sample. The sampling throughput can be easily increased taking into consideration that the diazepam extraction by sonication can be made simultaneously for a series of samples.

## Acknowledgements

Authors acknowledge the financial support of Ministerio de Educación y Ciencia (Project CTQ2005-05604/BQU, Feder) and Direcció General d'Investigació I Transferència Tecnològica de la Generalitat Valenciana (Project ACOMP06-161).

## References

[1] U.S. Drug Enforcement Administration. http://www.dea.gov/concern/benzodiazepines.html.
[2] J.E.F. Reynolds (Ed.), Martindale: The Extra Pharmacopoeia, 30th ed., The Pharmaceutical Press, London, 1993, p. 589.
[3] EC-U.S. Mutual Recognition Agreement Sectoral annex for pharmaceutical good manufacturing practices (GMPs). http://www.fda.gov/oia/ecmutual.htm.
[4] C. Ferreyra, C. Ortiz, J. Pharm. Biomed. Anal. 25 (2001) 493–499.
[5] M.F. Cholbi-Cholbi, J.J. Martinez-Pla, S. Sagrado, R.M. Villanueva-Camanas, M.J. Medina-Hernandez, J. Liq. Chromatogr. Relat. Technol. 27 (2004) 153–170.
[6] M. Bakavoli, M. Kaykhaii, J. Pharm. Biomed. Anal. 31 (2003) 1185–1189.
[7] S. Furlanetto, S. Orlandini, G. Massolini, M.T. Faucci, E. la Porta, S. Pinzauti, Analyst 126 (2001) 1700–1706.
[8] M.M. Coreira-dos-Santos, V. Famila, M.L. Simoes-Goncalves, Anal. Bioanal. Chem. 374 (2002) 1074–1081.
[9] M. Guadalupe-Garcia, A. Garcia, I. Gonzalez, Talanta 40 (1993) 1775–1779.
[10] C.F. Ferreyra, C.S. Ortiz, J. Pharm. Biomed. Anal. 29 (2002) 811–818.
[11] S. Jain, M. Tiwari, S.C. Chaturvedi, Indian Drugs 35 (1998) 696–699.
[12] S. Sadeghi, R. Takjoo, S. Haghgoo, Anal. Lett. 35 (2002) 2119–2131.
[13] J. Dolejsova, P. Solich, C.K. Polydorou, M.A. Koupparis, C.E. Efstathiou, J. Pharm. Biomed. Anal. 20 (1999) 357–362.
[14] J.V. de Julian, M. de la Guardia, Can. J. Spectrosc. 35 (1990) 44–50.
[15] The United States Pharmacopeia. Twentieth revision, United States Pharmacopeial Convention, Inc., Rockville, MD, 1980.
[16] A. Salari, R.E. Young, Int. J. Pharm. 163 (1998) 157–166.
[17] K. Pöllänen, A. Häkkinen, M. Huhtanen, S.P. Reinikainen, M. Karjalainen, J. Rantanen, M. Louhi-Kultanen, L. Nyström, Anal. Chim. Acta 544 (2005) 108–117.
[18] D. Lin-Vien, N.B. Colthup, W.G. Fateley, J.G. Grasselli, Infrared and Raman Characteristic Frequencies of Organic Molecules, Academic Press, London, England, 1991.
[19] E. Leonhardt, R. Stahl, Anal. Chem. 70 (1998) 1228–1230.