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Journal of Pharmaceutical and Biomedical Analysis
29 (2002) 1149–1152

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Short communication

FT-IR spectroscopy: a powerful tool in pharmacology

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Received 6 June 2001; received in revised form 26 November 2001; accepted 30 November 2001

Abstract

In the present work we report a Fourier Transform Infrared (FT-IR) analysis performed on rat encephalon samples in the CH–OH vibrational stretching region (2400–3800) cm^{-1}, in order to reveal the presence of a very diffuse commercial benzodiazepine: VALIUM®. The comparison between the spectral features of normal brain and the ones of samples with administrated substance has unambiguously showed that the CH stretching region seems not to suffer from any change for the pharmacological treatment, instead the OH band is strongly modified probably due to the presence of a new spectral contribution characteristic of diazepam molecule.

Keywords: FT-IR absorption; Medical physics; OH stretching region

1. Introduction

A noteworthy problem of recent pharmacological interest is undoubtedly represented by the possibility to reveal substances which are able to get over the haematic–encephalic barrier and hence eventually to recover or damage the brain. The monitoring of drugs distribution by means of traditional and usual methods can result quite difficult because of the necessity to use radioactive tracers.

Recently many different techniques have been employed for the diagnosis of disease states in the medical and/or pharmacological field, and among them vibrational spectroscopy has been successfully applied [1–4], due to the opportunity to perform a quantitative analysis on the various chemical composition and molecular structures of healthy and pathological tissues. In particular Fourier Transform Infrared absorption (FT-IR) spectroscopy has been employed extensively in this field in order to get information about the structural properties at molecular level [5–9]. Among the various advantages of FT-IR spectroscopy in this kind of study, one has to highlight the opportunity to analyse samples with thickness less than 1 mm and diameter less than 1 cm together with the reliability and the reproducibility of spectral data performed on human tissues

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0731-7085/02/$ - see front matter
PII: S0731-7085(02)00164-4

[10,11]. These latter are basic preconditions really difficult to obtain in the case of biological samples due to the complexity of this kind of material.

In the present paper we deal with a spectroscopic analysis carried out by FT-IR technique on rat brain, performed in the CH–OH stretching vibrational region (2400–3800) cm^{-1} in order to reveal the presence of a very common pharmacological substance, a benzodiazepine, in the brain. Essentially the main goal of the present work was to show how the FT-IR spectroscopic technique could be useful and powerful to study similar pharmacological problems.

2. Experimental

Five male Charles River rats, 180–200 g weight, were used and kept under standard conditions, with free access to food and tap water.

Thin slides of brain tissue, such as those required for optimal spectroscopic studies, about 30 μm thick, were obtained by using a standard cutting procedure, followed for routine morphological studies [12,13]. Initially, the samples of rat brain, were rapidly frozen in liquid N_2 and stored at -80 °C until the use. This cryofixing method has allowed to obtain reliable spectra, preventing the appearance of some unwanted contribution in the analysed spectral range, due to the presence of substances eventually used for fixing. Hence the samples were embedded in 'Optical Cutting Temperature (OCT) Medium' and then cut in slides ~ 30 μm thick by a freezer microtome (Reichert Jung) and laid on KBr pellets, which were transparent in the analysed wavenumber range. To make sure that the OCT compound did not give any unwanted contribution, we performed IR measurements on this compound. The layers were hence ready to be processed for the FT-IR analysis. Thanks to this method, the analysed samples were quite close to the vital conditions because any fixing liquid has not been used and furthermore their homogeneity in the whole extension of the layer allowed us to obtain spectra with high reproducibility.

As FT-IR absorbance measurements are concerned, the experimental data were collected on 15 normal brain slides cut from the five used rats and on the same number of samples with administrated diazepam ($C_{16}H_{13}ClN_2O$) in doses consistent with the animals weight, cut from the corresponding animals. The used instrument was a BOMEM DA8 FT-IR spectrometer, working with a globar lamp source, a KBr beamplitter and a DTGS/KBr detector (Deuterated Triglycine Sulphate detector), which is one of the most common detector used in the modern FT-IR spectrometers, working in the wavenumber range (200–7500) cm^{-1}. We studied, in the full set of samples, the CH–OH stretching region (2400–3800) cm^{-1}, automatically adding 100 repetitive scans for each run with a resolution of 4 cm^{-1}, to obtain a good signal-to-noise ratio, obtaining comparable and available spectra. The IR absorbance spectrum of pure diazepam in powder (Fig. 1) was performed and analysed as well. Each measurement was performed in dry atmosphere to avoid any unwanted contribution.

3. Results and discussion

Above all we performed IR absorbance measurements on the pure benzodiazepine in powder (see Fig. 1), to characterize its main vibrational bands in the analysed region.

In all the absorbance spectra collected in the case of rat brain, normal and treated with the drug, we can distinguish two clear regions: one

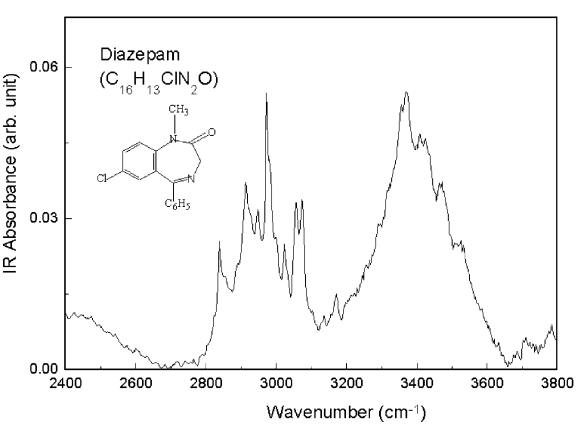

Fig. 1. Experimental IR absorbance spectrum of diazepam.

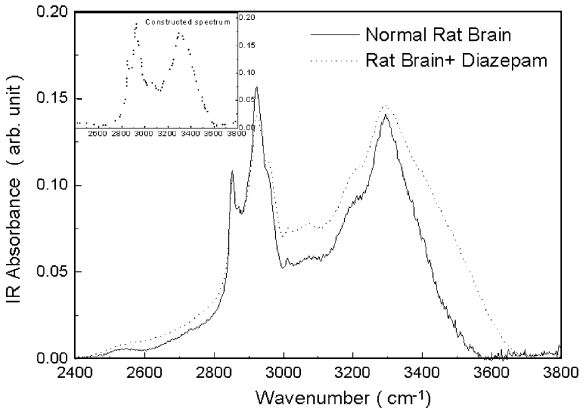

Fig. 2. Experimental IR absorbance spectrum of normal rat brain (—) and treated with diazepam (…). In the inset the *constructed* IR absorbance spectrum is shown.

that spreads from 2400 to 3000 cm^{-1}, characteristic of C–H stretching mode with different symmetries (CH_2, CH_3, etc.) and the other one in the range from 3000 to 3800 cm^{-1}, characteristic of O–H stretching vibration. Fig. 2 shows the experimental spectrum of normal brain together with the one corresponding to the brain sample treated with the diazepam. From a first inspection we can observe that, while the CH stretching band seems not to suffer from the pharmacological treatment, as we expect, the OH stretching band is clearly affected. In fact, in the experimental spectrum of treated brain there is a band centered at about 3500 cm^{-1} which is definitively absent in the absorbance spectrum of normal brain. To analyse the experimental data, we applied an usual best-fit [14] procedure, consisting of fitting the spectral features with theoretical profiles, based on the common criterium that for a given large band the number of sub-bands considered represents the minimum number of contributions and hence the minimum number of parameters above which the statistical error remains almost constant. Hence from the best-fit procedure, the presence of a sub-band centred at 3495.5 cm^{-1}, typical of the vibrational spectrum of diazepam (see Fig. 1), is confirmed. It has to be noticed that the other sub-bands, in the OH stretching region of the spectra, refer to molecules containing OH groups, including water, and that the centre-frequency lays in a spectral region with a great polarizability of the bands widely shifted respect to the free stretching OH groups ($\sim$ 3650 cm^{-1}) [15].

In conclusion, even if with this kind of analysis it is not possible to mark and characterize the different contributions to the OH vibrations, we can affirm doubtless that the involved OH groups strongly participated to H-bond interactions with macromolecules belonging to brain tissue. Furthermore, the presence of a new band centred at 3495 cm^{-1}, characteristic of this benzodiazepine, in the treated samples of brain, supports the occurrence that the drug, could get over the haematic–encephalic barrier, spreading in the brain tissue. To confirm this experimental evidence, we *constructed* a spectrum composed by the normal brain spectrum and the diazepam one (inset of Fig. 2). As we expected, the resulting spectrum seems to *mime* the experimental spectrum of treated brain.

4. Conclusions

We performed a FT-IR analysis on rat encephalon samples, in the CH–OH vibrational stretching region (2400–3800) cm^{-1}. The aim was addressed to reveal the eventual presence of a drug substance, in our case a commercial benzodiazepine, in the brain tissue, by a spectroscopic method. The comparison, in the fundamental O–H stretching vibration, between the spectral feature of normal brain and the one with administrated diazepam has unambiguously shown that the CH stretching region seems not to suffer from any change for the pharmacological treatment, as we expected, instead the OH band is strongly modified due also to the presence of a contribution which is characteristic of diazepam molecule.

Hence we can conclude that the applied spectroscopic analysis could be useful to characterize substances which are able, *or not*, to get over the haematic–encephalic barrier.

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