

DERIVATIVE UV SPECTROPHOTOMETRY USED FOR THE ASSAY OF DIAZEPAM FROM HUMAN BLOOD PLASMA

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Abstract

Therapeutic drug monitoring is a must when dealing with benzodiazepines, mainly at the beginning of the treatment and/or when changing the medication, in order to avoid accidental intoxications. Unfortunately, the assay methods are not handy in smaller medical facilities, as they require sophisticated devices, procedures and highly trained staff.

The present paper aims to develop a simple UV derivative method for the assay of diazepam from human plasma without previous chemical separation of the drug, using the fourth derivative of the spectra and the integrated area of the peak (valley) ranging from 306 to 333 nm. The method can be used for small diazepam concentrations (2 to 10 µg/mL), which are in the range of therapeutic blood concentrations reported for diazepam.

Rezumat

Monitorizarea terapeutică a tratamentului reprezintă o necesitate, mai ales în cazul benzodiazepinelor, la inițierea tratamentului sau la înlocuirea unuia din medicamente cu altul, pentru a evita intoxicațiile accidentale. Din nefericire, metodele curente de determinare cantitativă a concentrației de medicament în sânge nu sunt la îndemâna unităților medicale mici, deoarece necesită aparate și proceduri de analiză complexe, precum și personal cu înaltă calificare.

Lucrarea își propune să dezvolte o metodă simplă derivativă în UV pentru determinarea diazepamului în plasma umană fără procedură de separare chimică a analitului, folosind derivata a patra a spectrului probei și aria integrată a *peak*-ului pe domeniul 306-333 nm. Metoda poate fi folosită pentru determinarea concentrațiilor mici de diazepam (2-10 µg/mL), care se află în domeniul plasmatic terapeutic raportat pentru această benzodiazepină.

Keywords: diazepam, human blood plasma, UV spectrophotometry, derivative

Introduction

Since their introduction in the medical practice in the early 1960s the prescription of benzodiazepines increased constantly such that they are nowadays among the medicines most frequently administered in various diseases, due to their sedative, anxiolytic, hypnotic, anticonvulsivant effects. Despite the known addictive potential of benzodiazepines, they are an important class of drugs which cannot be replaced by other less harmful

pharmacological agents [1]. That is why the quantitative assay of these drugs is a central point in the pharmaceutical analytical chemistry, both in the case of medicines control and in therapeutical monitoring of the treatment with benzodiazepines in association with other drugs [2,3].

Diazepam is one of the oldest, while still being among the most often prescribed benzodiazepines [4,5]. The structure of benzodiazepines makes difficult their assay in mixtures and even more difficult in complex matrices such as biological fluids or tissues, mainly if low-cost methods are available. Therefore separation techniques generally have to be used to allow the robust detection of the analytes.

Derivative spectrophotometry (DS) is such a technique used to discriminate and allow the assay of certain analytes from complex mixtures or matrices *via* mathematical interpretation of the absorption signal. It is based on the so called *derivate spectra* which are generated from parent zero-order ones. The derivatisation of zero-order spectrum can lead to separation of overlapped signals and/or the elimination of background caused by presence of other compounds in a sample. Nowadays, this technique found application in many fields of analysis, mainly in the pharmaceutical, clinical and biochemical one as well as in inorganic or organic analysis [6,7].

The paper aims to develop a derivative UV spectrophotometric method for the assay of diazepam at low concentrations from plasma without chemical separation, to be further used in analytical toxicology in cases of polydrug intoxications.

Materials and Methods

Reagents

- Diazepam, pharmaceutical purity, was a gift from LaborMed Pharma – stock solution of 200 µg/mL in methanol
- Methanol, HPLC grade, from Sigma, was used as solvent in all experiments.
- Normal human plasma was obtained from the “C.T.Nicolau” Romanian Haematologic Institute

Devices

Cary 100 (Varian Inc.) UV-VIS spectrophotometer, Bio software package

Procedure

The human plasma was diluted 1:10 with double distilled water. Appropriate aliquots from the stock solution and of the diluted human

plasma to get the desired concentration of diazepam were pipetted in testing tubes and gently vortex-mixed for 5 minutes. A blank plasma sample was also prepared, containing the amount of methanol used for the samples. The UV spectra of the solutions were thereafter plotted against double distilled water in the range 200-400 nm, with a slit of 2 nm, at 200 nm/min speed. Previously, a background correction of the device was performed with water.

The zero-order spectra obtained were further derivatised (1-st to fourth derivatives) using the facilities of the software (Mathematics mode) and used in the analysis.

Results and Discussion

The zero-order spectra of the plasma containing 2.7, 4, 5 and 8 $\mu\text{g/mL}$ of diazepam are presented in Figure 1.

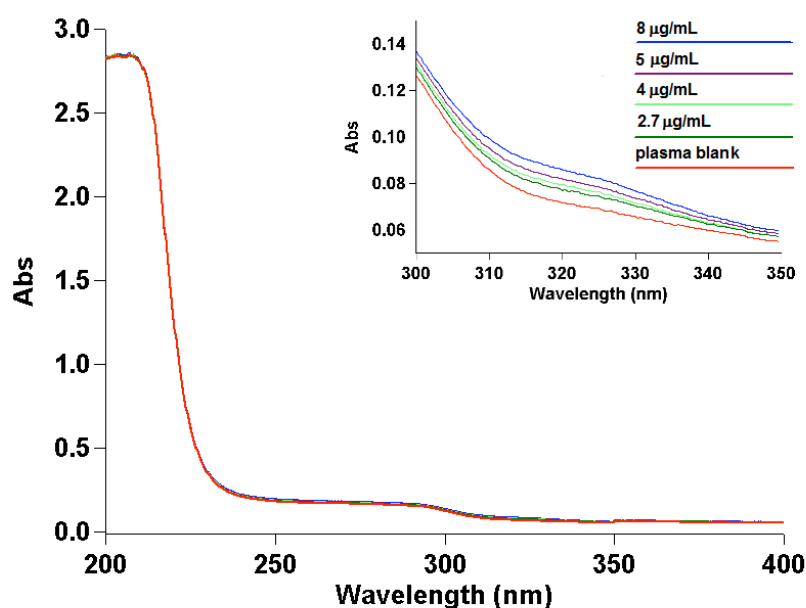


Figure 1.

Zero-order spectra of human plasma samples containing 2.7 to 8 $\mu\text{g/mL}$ of diazepam

Certain differences between the samples can be observed in the range 300-350 nm (inset in figure 1), but the differences between concentrations are too low to be interpreted in the common way (i.e. by measuring the absorbance at a certain level and plotting the regression line absorbance vs. concentration). Besides, the absorbance of the plasma spectrum from different patients can differ rather dramatically, and neither a

standard addition method can be used due to the same small differences in absorbance at the limit of the instrumental error (0.001 absorbance units).

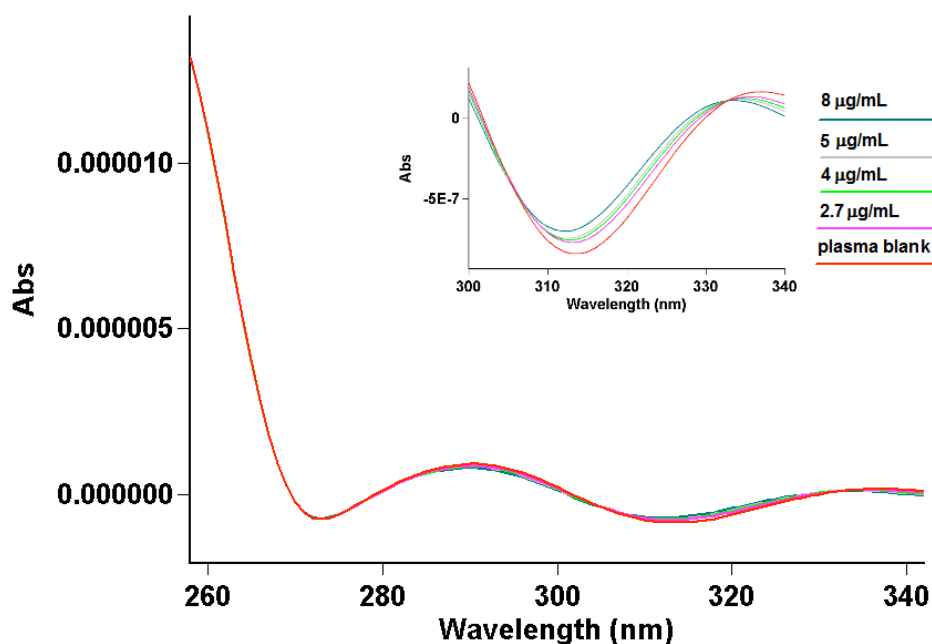


Figure 2.

The fourth derivatives of the spectra from Figure 1

We performed the first to fourth derivatives of the spectra to look for zones in which the differences between the spectra are interpretable (Figure 2 depicts the fourth derivative). As it can be seen, the derivatives are rather close to each other. However, we could identify in the region 300 – 330 nm of the fourth derivative a zone where the peaks (valleys) can be distinguished for each spectra and where the computational methods can be performed (i.e. a three point correction for the peak or an area of the peak can be computed, as all the spectra are intersecting at the same wavelength at one side and the other of the peak).

As can be seen from Figure 2, the fourth derivatives of the spectra intersect at 306 and 333 nm, and have a peak (valley) at 313 nm.

We performed a regression analysis on the data coming from repeated measurements performed on 5 replications of each of the following diazepam concentrations: 0 (plasma blank), 2.7, 4, 5, 8 and 10 µg/mL, using the peak value at 313 nm and the peak integrated value from 306 to 333 nm

as a function of diazepam concentration. The results are summarized in Table I.

Table I
Parameters used for the assay of diazepam from human plasma using the 4-th derivative of the UV spectrum

Parameter used	Equation (linear regression)	R ²	Standard error
Peak value ($\times 10^7$)	$Y = -7.9729 + 0.0964 \times x$	0.9588	0.8070
Peak area ($\times 10^6$)	$Y = -13.0781 + 0.3864 \times x$	0.9869	0.1797

Even if the correlation coefficients are quite close one to another (0.9792 for the peak value and 0.9934 for the peak area), the results are more reliable when using the area, as the influence of the background is canceled by the fact that the blank sample and the diazepam samples valleys are in the same range and the values on the ordinate scale are all of the same sign (all negative). Therefore we adopted this method for the quantification of diazepam in plasma without chemical extraction.

The values obtained for testing the accuracy of the method resulted in a mean recovery coefficient of 104.7% for samples containing 4, 5 and 6 $\mu\text{g/mL}$, which can be considered as acceptable for a simple and efficient method for therapeutic monitoring of diazepam in patients, taking into account that the therapeutic blood levels for diazepam range in the field 0.031-0.6 $\mu\text{g/mL}$ and lethal levels are above 34 $\mu\text{g/mL}$ (8). Of course, the method cannot replace methods as HPLC, GC, or LC-MS, but is far more simple, less time-consuming and less expensive than those.

Conclusions

We developed a simple and versatile method for diazepam quantitative assay in human blood plasma without separation of the drug from the biological matrix, using UV derivative absorption spectrophotometry. The method is based on the fourth derivative of the UV spectrum of blood plasma containing small amounts of diazepam (ranging in the field 2-10 $\mu\text{g/mL}$), by integrating the area of the peak (valley) between 306 and 333 nm, where the derivatives are dependent of diazepam concentration. The method can be useful to monitor the therapeutic blood level of diazepam in order to avoid accidental intoxication with diazepam, mainly at the beginning of the treatment. The method cannot replace already classical methods such as HPLC or GC-MS, but is far more easier to perform.

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