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DETERMINATION OF THE ACTIVE INGREDIENT (5-ETHYL-2'-DEOXYURIDINE) OF THE OINTMENT 'REVIDUR' BY UV AND NIR SPECTROSCOPY

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Abstract

UV spectrophotometric and NIR spectroscopic methods were developed for determining the active ingredient (5-ethyl-2'-deoxy-uridine) in the antiviral ointment (Revidur).

UV spectrophotometric measurements were carried out at 263 nm with solution of the ointment in absolute ethanol prepared by ultrasonication. Calibration solutions contained the matrix. The standard error of the method was 0.016%, the correlation coefficient of the calibration curve was 0.999 in the range 0.25 to 2.5% drug content.

Two NIR methods are described in the present work. In one of the methods, NIR reflectance spectra were taken of solutions and concentration determinations were carried out based on the second derivative of the spectra at 1166 nm.

The correlation coefficient was equal to that calculated for the UV method, and the standard calibration error was slightly higher, 0.05%. The advantage of the method is its quickness if calibration data are available.

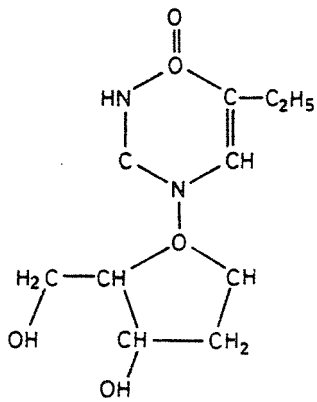
The simpler method developed seems to be suitable for the determination of the drug directly in the ointment without any pretreatment. In the is method, the second derivative of the NIR reflectance spectra measured at 2466 and 1528 nm are used. However, due to inhomogeneity problems with the calibration samples resulting in a standard calibration error one order of magnitude higher than that for the solution technique, we consider our results with the direct method to by only preliminary onesand intend to continue our studies.

Keywords: determination of 5-ethyl-2'-deoxyuridine

[1]EGIS Pharmaceutical Work, Budapest, POB 100, 1475
[2]REANAL Fine Chemicals, Budapest, POB 54, 1441

Introduction and Literature Survey

Chemical structure, formula and name of the drug:



$C_{11}H_{16}N_2O_5$
Uridine, 2 – deoxy – 5 – ethyl –
(15176 – 29 – 1)
Edoxudine

Structural studies of the compound by NMR and CD spectroscopy were published in 1973 (CLEVE, HOYER, SCHULZ G. and VERBRUEGGEW) and 1975 (KEMIN, I. EKIEL, and SHUGAR). Separation from biological matrix by HPLC (CSARNYI, SZABOLCS, VAJDA and "OTVÖS 1979), ion-pair chromatography (HEMPEL, 1982), GC (GARZE, ALEXANDER and TILL 1980), TLC and LC (KAUL and HEMPEL, 1985) have also been dealt with in the literature.

However, the determination of the drug in pharmaceutical preparations appears to have not been described yet. The preparation studied in the present work is an emulsion-type ointment with antiviral effect produced by REANAL (Budapest, Hungary). During manufacture, the aqueous solution of the drug is homogenized in the matrix. The drug content of the ointment is controlled by a method elaborated in the manufacturer's laboratory. The procedure consists of the following steps:

1. Extraction of the organic matrix with chloroform after addition of water and zinc chloride.
2. Separation of the organic and aqueous phase.
3. Preparation of a calibration graph using aqueous solutions containing the drug in different and $ZnCl_2$ in constant concentration after measuring the absorbance at 267 nm.
4. The drug transferred in the aqueous phase is determined by means of the calibration graph.

In the present work, a method has been worked out for the determination of the active principle in the ointment 'Revidur' which involves

no extraction step and applies calibration measurements in the presence of the ointment matrix to eliminate matrix effects. The composition of the ointment is as follows (for 10 g total weight):

drug: 0.1 g;
sorboxaethenum stearinicum 0.40 g,
paraffinum liquidum 0.40 g,
alcohol cetylstearylicus 1.20 g,
vaselinum album 2.00 g,
solution conservans 0.20 g,
aquadestillata 5.70 g.

Determination of the Drug by UV Spectrophotometry
Experimental Conditions and Solution

The drug content was determined in absolute ethanol solution. By sonication sall the components of the ointment were dissolved in absolute ethanol which could not have been achieved otherwise. A series of calibration solutions was prepared by mixing the solution of the matrix and that of the drug in appropriate proportions.

Ointment samples were dissolved in absolute ethanol by sonication.

Absorbance measurement was carried out by a Zeiss Specord UV-VIS spectrophotometer.

— The solution of the matrix: 0.1 g matrix was weighed on an analytical balance, transferred to a 100 cm^3 volumetric flask with 80 cm^3 absolute ethanol and sonicated for 4 min. The solution thus obtained was filled up to the mark with absolute ethanol.
— Sample solution was prepared similarly, using ointment portions depending on the expected drug concentration (0.1 g in the case of 1% and 0.08 g in the case of 2.5% drug content).
— Drug stock solution
0.05 g of the drug was dissolved in absolute ethanol similarly to the above solutions.

Calibration solutions were prepared as given in *Table 1.*

Results and Discussion

The UV spectra of the solution of the matrix and of the two ointment samples were taken in the range of 200–300 nm and it was found that the matrix components did not interfere in the determination of the drug if the solution of the matrix was used as reference solution (*Fig. 1*).

The spectra of the calibration series are shown in *Fig. 2.*

Table 1
Data of the series of calibration solutions for the determination of the drug in the ointment Revidur by UV spectrophotometry

Drug stock solution, cm^3	0.05	0.10	0.20	0.30	0.50
Solution of the matrix, cm^3	9.95	9.90	9.80	9.70	9.50
Drug concentration (in % of the matrix)	0.25	0.50	1.00	1.50	2.50

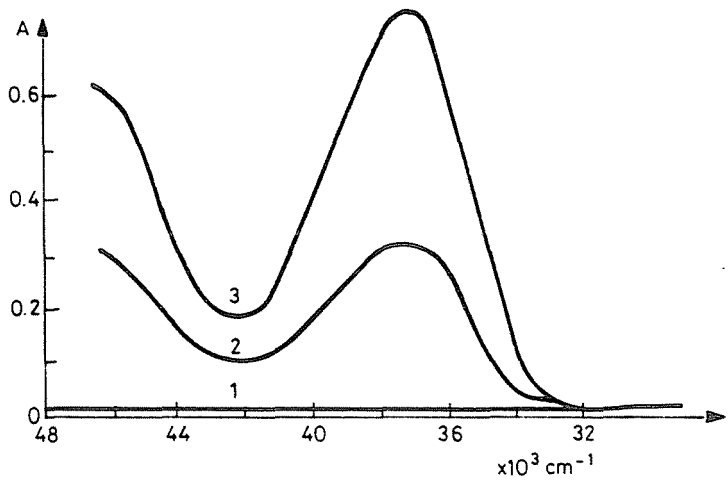


Fig. 1. UV spectra of the solutions of the ointment matrix and of two Revidur ointment samples in absolute ethanol. 1: matrix; 2: Revidur sample with 1% drug content; 3: Revidur sample with 2.5% drug content

five parallel series were prepared, each series consisting of five members.

The parameters of the equation describing the concentration dependence of the absorbance measured at 263 nm, the correlation coefficient and the standard error of the calibration (for interpretation see the next chapter) were calculated:

$$A_{263\,\mathrm{nm}} = -0.008 + 0.351c \quad [\%],$$

$$r = 0.999,$$

standard calibration error=0.016 [%].

The above equation was used for determinig the drug in various preparations and the results were always close to the values expected.

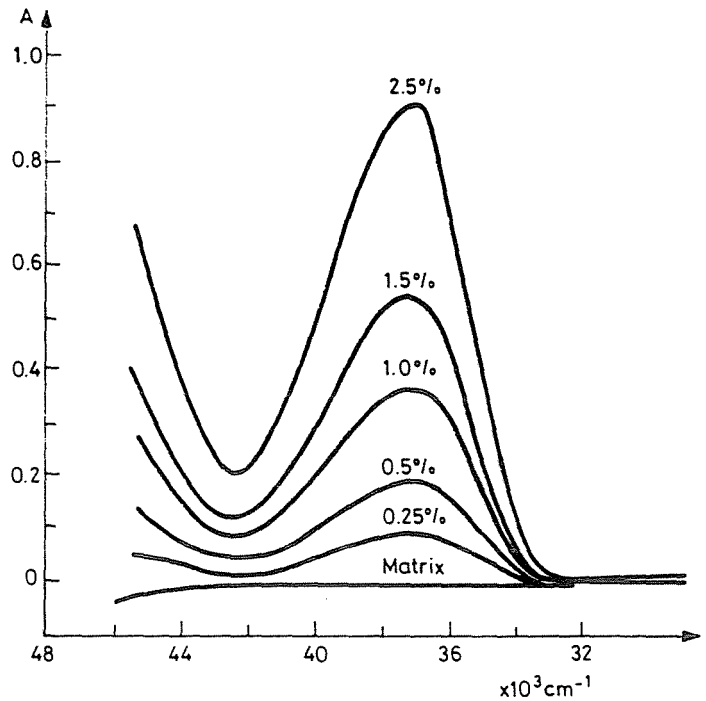


Fig. 2. UV spectra of the calibration solutions

The recovery of added drug was also studied. Some of the results are as follows:

Theoretical concentration, %	1.60	2.10	2.59
Measured concentration, %	1.58	2.08	2.56

Determination of the Drug by NIR Spectroscopy

Determination in Absolute Ethanol Solution

Experimental Conditions and Solutions

Calibration solutions were prepared as described in Chapter 1. The drug content of the series members in % of the matrix were: 0.25, 0.50, 1.0, 1.5 and 2.5. In preliminary experiments, NIR spectra of the calibration solutions were taken against the internal reference of the instrument. The latter gave better results.

The measurements were made using a Pacific Scientific NIR instrument type 6250.

Results and Discussion

Three spectra were taken of each calibration solution and the computer program of the instrument processed the second derivative of the averaged spectrum to find the wave length at which there is correlation between the spectrum or its second derivative and the concentration of the drug and to determine the parameters of the calibration curve, correlation coefficients and other statistical parameters.

The reflectance spectrum and its second derivative for a calibration solution are shown in *Fig. 3*, the calibration curve calculated for the second derivative at 1166 nm, with the standard deviation of the deviation of concentrations determined based on the calibration curve from actual concentrations are shown in *Fig. 4*.

The value of the correlation coefficient (r) reflects a close correlation between optical and chemical data.

Direct Determination from Ointments (Preliminary Experiments)

The aim of our further NIR studies was to simplify the technique by eliminating both the extraction and dissolution, i. e. to carry out the determination directly in the ointment which is a viscous liquid.

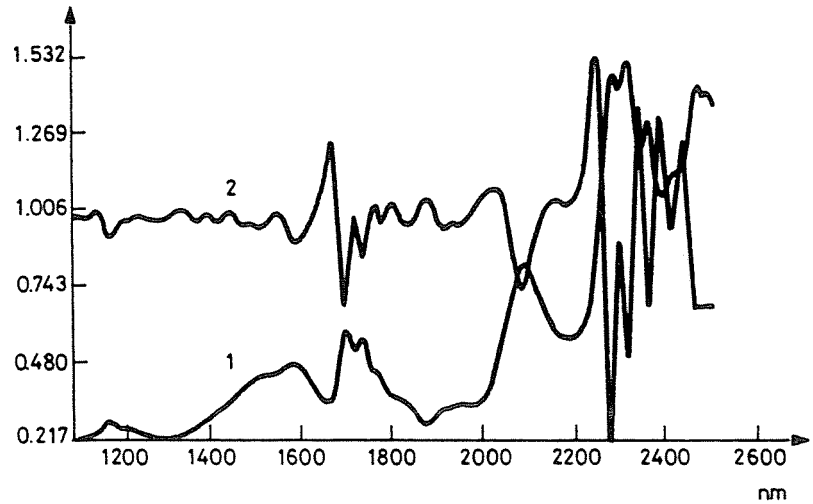


Fig. 3. NIR reflectance spectrum (1) and its second derivative (2) taken of a calibration standard solution

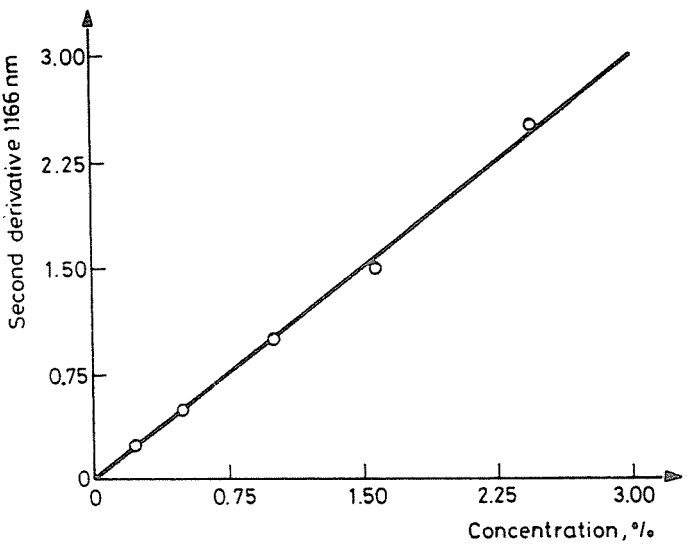


Fig. 4. Calibration curve at 1166 nm. Standard error of calibration: 0.05%, r=0.999

Experimental Conditions

The preparation of the calibration series was problematic, as no ointment samples with different drug content were available. So we prepared calibra-

Table 2
Data of the calibration series for the direct determination of the drug in the ointment by NIR spectroscopy

No. of calibration sample	1	2	3	4	5
Concentration of the drug, %	4.0	2.0	1.0	0.5	0.1
Weight of matrix	9.6 g	5.0 g	5.0 g	5.0 g	4.0 g
Weight added	0.4 g drug	5.0 g ointment with 4% drug	5.0 g ointment with 2% drug	5.0 g ointment with 1% drug	1.0 g ointment with 0.5% drug

tion samples by gradual dilution from the matrix and the active ingredient of the ointment with known concentrations in amounts shown in *Table 2*. The samples thus prepared were homogenized in an agate mortar. The concentrations of the calibration samples were controlled by the UV spectrophotometric method given in Chapter 2. Calibration was carried out in the concentration range 0.2–4%. NIR reflectance spectra and their second derivatives were taken as described above against the internal reference of the instrument.

Results and Discussion

The instrument processed the average from three spectra. The reflectance spectrum and its second derivative of a calibration sample is shown in *Fig. 5*, the calibration curve produced by the instrument based on the second derivatives at 2466 nm and 1528 nm in *Fig. 6* and *Fig. 7* respectively, along with the standard error and correlation coefficient.

Using the calibration curves, some Revidur ointment samples were analyzed for the active ingredient under the same experimental conditions. The results obtained were close to those expected through determination by the solution method UV and NIR spectroscopy.

The method for the direct drug determination from the ointment needs further refinement, since inhomogeneity problems arose in the calibration samples reflected by the correlation coefficient and standard calibration error values at both wavelengths. In the present paper we intended to publish the results of our preliminary experiments with the direct NIR determination.

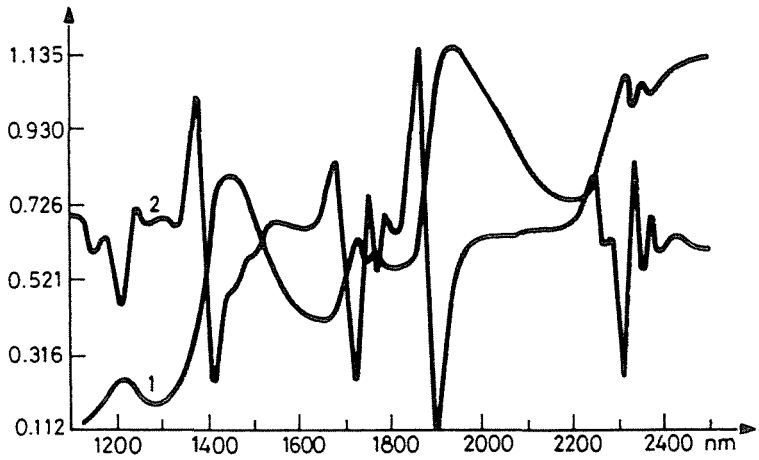


Fig. 5. NIR reflectance spectrum (1) and its second derivative (2) of an ointment calibration sample

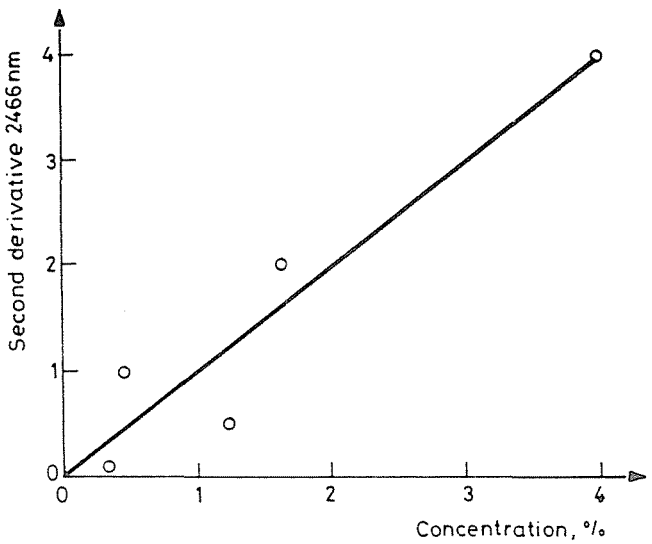


Fig. 6. Calibration curve for 2466 nm, standard error of calibration: 0.50%, r=0.955

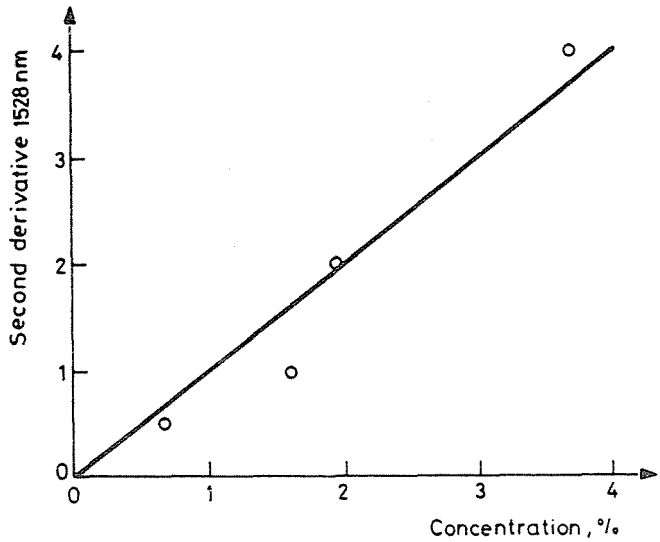


Fig. 7. Calibration curve for 1528 nm, standard error of calibration=0.47%, r=0.965

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