# DETERMINATION OF TETRACYCLINES IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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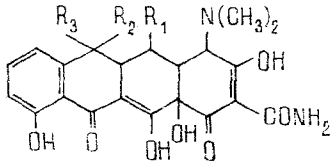
A new method for the separation and quantitative determination of certain antibiotics of the tetracycline series in blood plasma by high-performance liquid chromatography on a column filled with Partisil PXP 5 silica gel is described.

In recent years, high-performance liquid chromatography has found wide use in the analysis of drugs. This method is used both for evaluating the purity of pharmacological preparations and for determining them in biological materials (plasma, urine, tissues). High-performance liquid chromatography permits the separation and identification of trace amounts of substances of different chemical natures in a very short time.

Several methods of separating the most important clinical antibiotics of the tetracycline series on specific supports based on modified silica gel have been described [1-3]. However, it is impossible to regard the analytical procedure as having been developed definitively, since new variants of it are appearing in the press, [4-6], which indicates methodological difficulties arising in the chromatographic separation of the tetracyclines. It may be assumed that these difficulties are due to features of the structure of the tetracycline molecule (see formula) — their polycyclic and polyfunctional nature and their capacity for forming chelate complexes with metals.




| | $R_1$ | $R_2$ | $R_3$ | |
|---|---|---|---|---|
| I | OH | $CH_3$ | OH | Oxytetracycline |
| II | OH | $=CH_2$ | — | Methacycline |
| III | OH | $CH_3$ | H | Doxycycline |
| IV | H | $CH_3$ | OH | Tetracycline |

The properties of the tetracyclines that have been described require, on the one hand, a strict standardization of the conditions for their separation and, on the other hand the standardization of the supports themselves. Thus, we have been unable to reproduce the separation of the tetracyclines on the modified silica gel Spherisorb ODS, comparable in properties with the support μ-Bondapak $C_{18}$, on which the separation of the antibiotics has been achieved previously [2]. It is obvious that in this case the sorbents differ with respect to the degree of silylation of the silica gel, and the presence of a certain amount of unreplaced Si—OH groups exerts a serious influence on the reversed-phase chromatography of the tetracyclines. Furthermore, in order to suppress the interaction of the basic $(CH_3)_2N$ group of the tetracyclines the OH groups of chemically modified silica gel requires the use of a mobile phase with pH$\leqslant$1.5, which may cause the complete breakdown of the support. Consequently, we have renounced reversed-phase chromatography and as the support for separating the tetracyclines we have selected the unmodified silica gel Partisil PXS 5.

To decrease complex-formation by the tetracyclines with metals, leading to the appearance of broad, diffuse, peaks on the chromatograms [1], we saturated the mobile phase with ethyldiaminetetraacetic acid (EDTA). The separation of the mixture of antibiotics was considerably improved on the addition of 0.5% of acetonitrile to an eluent with pH 1.0. In this case, the tetracyclines are characterized by comparative short retention times: oxytetracycline 7.5 min, methacycline 8.1 min, doxycycline 9.6 min, tetracycline 17.8 min (Fig. 1A). Under these conditions, chlorotetracycline is not departed from tetracycline and only a small inflection on the right-side of the tetracycline peak can be seen on the elution curve.

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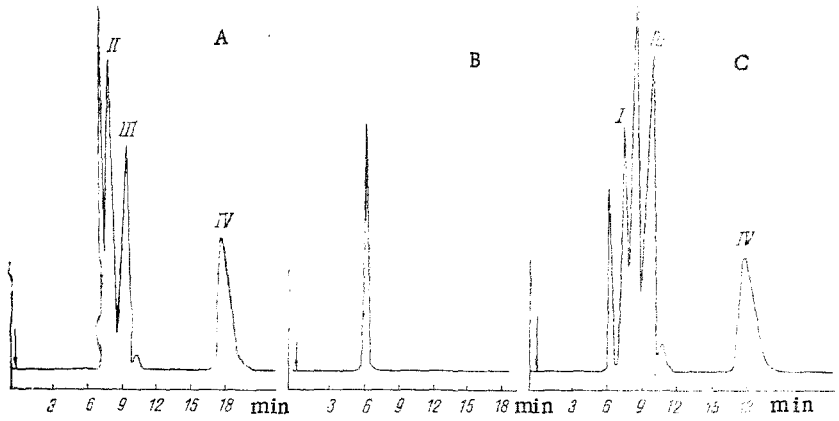


Fig. 1. Chromatographic analysis of the tetracyclines on Partisil PXs 5: A) standard mixture of tetracyclines: I) oxytetracycline; II) methacycline; III) doxycycline; IV) tetracycline; B) extract of the control plasma (without antibiotics; C) extract of tetracyclines from the plasma. For separation conditions see the Experimental part.

To decrease the residence time of tetracycline and to obtain a narrow and sharper peak of this antibiotic it is possible to use gradient elution with an increase in the concentration of acetonitrile in the mobile phase after the issuance of the doxycycline.

In the course of the investigation we detected no basic differences in the behaviors of the antibiotics on columns with the unmodified silica gel of various firms. Calibration of the instrument for each concentration of antibiotic (0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 μg) was performed not less than three times, and on the basis of the results a graph was plotted of the dependence of the height of the peak on the concentration of the antibiotic, from which the concentration of medicinal agent in the plasma was determined.

The second problem arising in the analysis of the tetracyclines is connected with difficulties in their quantitative extraction from blood plasma, which is due to the sorption of the antibiotics by the plasma proteins. To increase their yield from the plasma, use is frequently made of extraction by organic solvents in the presence of so-called carrier substances [4, 7], with which, under certain conditions, tetracycline forms complex compounds and readily passes into an organic phase. However, many of these carriers are difficultly accessible substances and by no means do they always ensure the complete extraction of the antibiotics from the plasma. In view of the polyphenolic nature of the tetracycline, as carrier we have used a common cationic detergent — cetyltrimethylammonium bromide. The use of this carrier ensures the extraction of the majority of antibiotics investigated from an aqueous into an organic phase, with the exception of oxytetracycline. This is obviously due to its higher polarity.

As can be seen from Fig. 1B and C, the substances of plasma (control experiment without tetracycline), that pass on extraction into the ethyl acetate have short retention times on the column and in practice do not interfere with the determination of the antibiotic. The proposed method permits the determination of about 0.3 μg of each of the antibiotics in 1 ml of blood plasma.

## EXPERIMENTAL

The chromatographic separation of the antibiotics was carried out on an Altex liquid chromatograph with an 8-μl flow-through cell and a 20-μl injector. To detect the antibiotics we used a Hitachi variable wavelength spectrophotometer. A column 4.6 × 250 mm was filled with a silica gel Partisil PXS 5 or Lichrosorb Si 60. The samples of antibiotics (tetracycline, chlortetracycline, oxytetracycline, methacycline, and doxycycline) were kindly provided by Professor A. D. Kuzovkov. The EDTA and $NaH_2PO_4$ were of kh.ch. ["chemically pure"] grade, the ethyl acetate was spectrally pure, and the acetonitrile was the Merck product.

Chromatography of the Tetracyclines. A new column containing the silica gel Partisil PXS 5 was washed with water, with 50% acetonitrile, with water, and with 0.1 N $NaH_2PO_4$ brought to pH 1.0 with nitric acid and saturated with EDTA, and, finally, it was equilibrated with a mobile phase consisting of 99.5% of 0.01 N $NaH_2PO_4$, pH 1.0, saturated with EDTA, and 0.5% of acetonitrile. Elution was performed at the rate of 1 ml/min at a sensitivity of 0.01, the detection of the tetracycline being performed at 355 nm.

Extraction of the Tetracyclines from Blood Plasma. To 1 ml of plasma was added a standard solution of tetracyclines and 50 μl of an aqueous solution of cetyltrimethylammonium bromide (5 mg/ml), the mixture was carefully stirred, and the antibiotics were extracted with ethyl acetate (3 × 3 ml) for 5 min. The suspension was separated by centrifugation, the ethyl acetate layer was evaporated in vacuum, the dry residue was dissolved in 0.5 ml of 0.01 N HCl, and an aliquot (20 μl) was deposited directly on the column of the chromatograph.

## SUMMARY

1. A new method is proposed for separating the antibiotics tetracycline, oxytetracycline, methacycline, and doxycycline on silica gel by high-performance liquid chromatography.

2. A new variant of the extraction of tetracyclines from plasma into an organic phase with the aid of a carrier — cetyltrimethylammonium bromide — has been developed. The method permits the determination of about 0.3 μg of the antibiotics in 1 ml of blood plasma.

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# INFLUENCE OF AMINES ON THE CHANGE IN THE SPECTRAL PROPERTIES OF ALKALINE SOLUTIONS OF SODA LIGNIN AND THE TRANSFORMATION OF SULFATE CELLULOSE ON PHOTOIRRADIATION

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It has been shown that in the irradiation with light of alkaline solutions of soda lignin and suspensions of sulfate cellulose additions of ethylenediamine, monoethanolamine, and hydrazine lead to a decrease in the optical density in the visible region of the absorption spectra of the samples studied.

The yellowing of lignocellulose materials under the action of sunlight is connected with photochemical reactions leading to the degradation of the components of wood. A leading role in this process is played by the chromophoric groups of the lignin, which are formed as the result of its oxidation and photochemical decomposition. The chromophores formed in the cellulose and the extraction substances amount to only 10% and less than 1%, respectively, [1]. The absorption of lignin and technical celluloses in the visible region of the spectrum is due, in the opinion of many workers, to the presence of chromophores containing carbonyl groups conjugated with aromatic rings, quinone methide groupings and diphenyl structures, and also α,β-conjugated double bonds [2].

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