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COMPARISON OF SEVERAL TYPES OF POLY (STYRENE-DIVINYLBENZENE) COPOLYMER FOR THE ANALYSIS OF OXYTETRACYCLINE AND DOXYCYCLINE BY HPLC

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ABSTRACT

The comparative study for the analysis of oxytetracycline hydrochloride and doxycycline polyphosphate by high performance liquid chromatography using several types of poly(styrene- divinylbenzene) column packings is described. The main compound is separated from potential impurities. The separation obtained on above mentioned packing materials of different origin is comparable.

Introduction

Oxytetracycline (OTC) epimerizes to 4-epi-oxytetracycline (EOTC), but the epimerisation rate is low (1,2). The acid degradation results in the formation of anhydro-oxytetracydine (AOTC). This anhydro derivative rearranges in acid hydroxylic media to two isomers, a-apo-oxytetracycline (a-APOTC) and b-apoxytetracycline (b-APOTC) (1-3). Hochstein et al described another related compound, 2-acetyl-2-decarboxamido-oxytetracycline (ADOTC) (4). It is a fermentation impurity which is practically inactive therapeutically. Tetracycline (TC) is also described as a fermentation impurity of OTC (1,5). EOTC, OTC, ADOTC, TC, AOTC, a-and b-APOTC are successfully separated by the HPLC method described.

Doxycycline (DOX) is prepared from OTC (6, 7). Metacycline (MTC) is an intermediate product and 6-epidoxycycline (6-EDOX) is a much less active by-product. Therefore, MTC, 6-EDOX and to a smaller extent OTC may be present as impurities.

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DOX does not easily epimerize, and due to absence of a hydroxyl group at C-6, DOX does not show acid degradation. This means that unlike OTC, DOX should not be analysed for anhydroderivatives. 6-EDOX can also epimerise to 4, 6-epidoxyccline (4,6-EDOX). 2-Acetyl-2- decarbaxamidodoxycycline (ADDOX) can be formed from the corresponding ADOTC if it is present in the starting material. OTC, 4,6-EDOX 4-EDOX, MTC, 6-EDOX, ADDOX and DOX are well separated by the method described.

The HPLC methods for OTC, described in literature, use ion exchange of reversed phase materials. The HPLC methods which can be used for the purity control of OTC were published in the last decade (8-10).

Some papers describe the determination of DOX by HPLC in biological sample: while others mention systems to separate DOX fr*om* other tetracyclines. Only fen papers mention HPLC methods suitable for purity control of DOX (11-15).

Most recently in our laboratory HPLC methods were developed for the purity control of OTC, DOX, chloretetracycline (CTC) and tetracycline (TC) (16-21). Present study for the comparison of several poly (styrene-divinylbeozene) copolymer (PSDVB) stationary phases by isocratic HPLC of DOX and OTC is based upon methods already described (16-18).

Experimental

Solvents and reagents

Tertiary butane! (t-BuOH) and tetrabytylammonium hydrogen sulphate were obtained from Janssen Chimica, Beers; Belgium. Other reagents were of A.R. grade (Merck, Darmstadt, F.R.G.). Water was glass distilled freshly.

HPLC apparatus and operating conditions

The HPLC apparatus consisted of a three solvent delivery system SP-8700-XR (Spectra Physics, San Jose, CA, U.S.A.), an injector Model CV-6-UHPa-N60 (Valco, Houston, TX, USA.) equipped with a 20 µ1 loop, a fixed wavelength detector (254 nm) Model 440 (Waters Associates, Milford, MA, U.SA.) and a Model 3390 A integrator (Hewlett Packard, Avondale, PA, U.S.A.). The columns (250 mm x 4.6 were packed in our laboratory with different PSDVB materials by the method already described (22).

The column was immersed in a water bath at 60°C and the flow rate was kept at 1.0 ml/min. Each evening the pump was washed with methanol-water (50:50), but the column was never washed. The back pressure was between 1000 and 2500 p.s.i.

Mobile phases

Mobile phases with different amount of t-BuOH were used. The required amounts of 5-BuOH were weighed and rinsed into a volumetric flask with water. All mobile phases contained 10% v/v of 0.2 M potassium hydrogen phosphate buffer pH 8.0, 5% v/v of 0.02 M tetrabytylammonium hydrogen sulphate (TBA) and sodium edetate (EDTA) (10% v/v of 0.0001 M for OTC and 10% v/v of 0.01 M for DOX). During preparation of the latter two solutions, the pH was brought to 8.0 with sodium hydroxide solution. The volume was made up with water. The mobile phases were degassed by sonication.

Reference substances

Reference substances of TC, EOTC, a-and B-APOTC and a OTC standard were prepared in our laboratory and are now available from Janssen Chimica, Beerse, Belgium. Reference substances of MTC, 6-EDOX and DOX were obtained from the European Pharmacopoeia laboratory, Strasbourg, France. 4,6-EDOX or 4-EDOX were prepared in solution by partial epimerisation of 6-EDOX or DOX (14).

Sample preparation

For the purpose of comparison, commercial samples of OTC hydrochloride and DOX polyphosphate were used. About 20 mg of the samples were weighed, dissolved and diluted to 20.0 ml with 0.01 N hdyrochloric acid.

Stationary phases

PSDVB stationary phases of different origin were examined: (i) PLRP-S 8 urn (Polymer Laboratories, Church Stretton, Shropshire, U.K.); (ii) PAP-1 10 urn (Hamilton, Reno, NV, U.S.A.); (iii) ROGEL 7-9 urn (RSL-Alltech Europe, EKE, Belgium).

Results and Discussion

Comparison with doxycycline

The preliminary investigations were carried out on PLRP-S using mobile phase containing t-BuOH as an organic modifier. The influence of the pH of the mobile phase containing 6% t-BuOH is shown in figure 1. Other experimental details have al-ready been published (14, 18). It seems that the quality of separation at pH 8.0 or pH 7.0 is comparable. At pH 7.0 however the peaks are less symmetrical. It was observed that here 4-EDOX eluted before 4,6-EDOX. The time needed for complete elution is somewhat longer as well. At pH 8.0, the separation between OTC and 4,6-EDOX was less satisfactory but still sufficient since those impurities are at most present in a very tiny amount. At pH 9.0, 4-EDOX is eluted close to 6-EDOX. MTC, 4-EDOX, and 6-EDOX are not separated from each other. Finally pH 8.0 was retained as the pH of choice where OTC, 4,6-EDOX, 4-EDOX, MTC, 6-EDOX and DOX are separated completely. At pH 8.0, 2-acetyl-2-decarboxamidodoxycycine (ADDOX) is sufficiently separated from DOX to allow the quantitation of 0.2% or more.

Typical chromatograms of DOX polyphosphate obtained on PLRP-S, PRP-1 and ROGEL are shown in figure 2. The three packing materials showed comparable separation of potential impurities from DOX. On ROGEL 4,6-EDOX is better separated from 4-EDOX as compared with PLRP-S or PAP-1. DOX polyphosphate samples contain more 4-EDOX than DOX hyclate or DOX base samples. This is due to the fact that the epimerisation is catalysed by phosphate ions (1). At the other hand the potential impurity ADDOX was not found to be present in the DOX polyphosphate samples examined.

It was also observed that for better separation and good peak symmetry, it is necessary to immerse the column in a water bath. The use of a water jacked does not always allow adequate heating of the top of the column and can cause peak distortion.

Comparison with Oxytetracycline

A pH 8.0, already used for DOX, was retained as a proper choice. For isocratie HPLC of tetracycline (TC) pH 9.0 was retained as a pH of choice (21). The influence of pH and the influence of organic modifier has already been discussed in detail (16).

The mobile phase was prepared in the same way as mentioned above. However, the concentration of EDTA in the mobile phase was decreased from 10% v/v 0.01 M to 10% v/v 0.0001 M. This was necessary when gradient elution was applied, since higher concentration caused base line problems (16). For reasons of uniformity, this lower concentration was also used in isocratic experiments.

Chromatograms of OTC, obtained by isocratic elution on several PSDVB materials, are shown in figure 3. The general elution pattern was similar but there was some difference in quality of separation, which seems to be correlated with the particle size, since the smallest particles gave the better separation. As for DOX, more of the organic modifier t-BuOH was necessary to obtain comparable retention times on the ROGEL column. The difference in quality of separation was primarily observed for the pair OTC-ADOTC. The separation of this pair was better on ROGEL and on this column, another impurity of unknown origin was also separated right after BAPOTC. The chromatograms show clearly that gradient elution has to be used if better separation in the first part of the chromatograms has to be realised and the peaks have to be eluted within an acceptable analysis time. A reduction of the anal time will also improve the detectability of the small impurities, eluted at the end of chromatogram.

It can be concluded that the separation pattern is comparable for the three PSDVB stationary phases examined and which are the most important on the market. In comparison with the classical silica gel based reversed phases this is an important advantage. This improves the applicability of methods based on PSDVB materials in different laboratories and thus the ruggedness of the method.

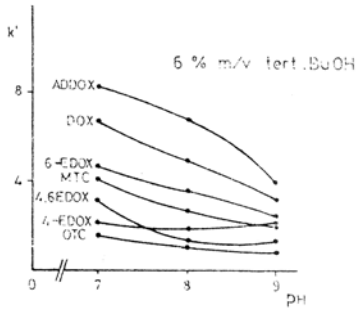

Fig. 1: Influence of pH on the separation of doxycycline and related substances.
Column: PLRP.S 8um (250mm x 4.6 mm)
Mobile phase: t-BuOh (6.0 g) - 0.2 M phosphate buffer pH y (10.0 ml) - 0.02 M tetrabutylammonium sulphate pH y (5.0 ml) .0.1 M sodium edetate solution pH y (1.0 ml) - water (up to 100.0 ml).
pH = y as indicated in figure
Flow rate: 1.0 ml/mm; detection: UV at 254 nm; temperature: 60ºC.
OTC = Oxytetracycline, 4-EDOX = 4-epidoxyclycline, 4,6-EDOX = 4,6-epidoxycyclinc, MTC = metacycline, 6-EDOX = 6-epidoxycyciine, DOX = doxycycline, ADDOX = 2-acetyt-2-decarboxamidodoxycycline.

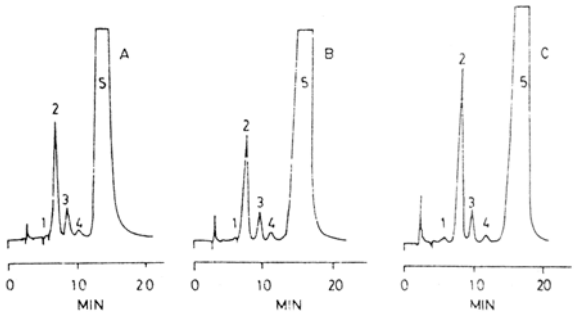

Fig. 2: Comparison of several poly (styrene-divinylbenzene) copolymers by isocratic HPLC of doxycycline poly-phosphate.
Columns: (250mm x 4.6 mm)
APRP-1 (10 um) x = S.8g
BPLRP-S (Sum) x = 5.8g
CROGEI1 (7-9um) x = 7.6g
Mobile phase: t-BuOH (x g) - 0.2 M phosphate buffer p11 8.0 (10.0 ml) - 0.01 M sodium edetate pH 8.0 (10.0 ml) - 0.02 M tetrabutylammonium sulphate pH 8.0 (5.0 ml) - water (up to 100.0 ml>.
Flow rate: 1.0 ml/mm; detection: UV at 254 nm; temperature: 60^0C.
1 = 4,6-epidoxycycline, 2 = 4-epidoxycycline,
3 = metacyclline, 4 = 6-epidoxycycline, 5 = doxycycline.

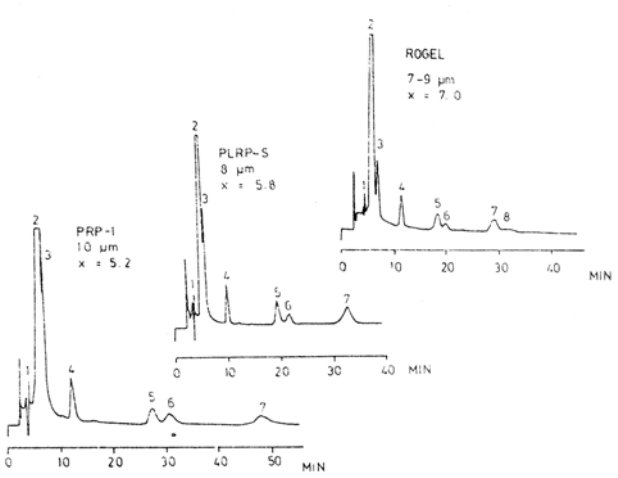

Fig. 3: Comparison of several poly (styrene-divinylbenzene) copolymers by isocratic HPLC of oxytetracycline hydrochloride.
Columns: (250mm x 4.6 mm)
PRP-1 x=5.2g
PLRP-S x=5.8g
ROGEI x=7.0g
Mobile phase: t-BuQH (x g) -0.2 M phosphate buffer pH 8.0 (10.0 ml) -0.02 M tetrabutylammonium sulphate solution pH 8.0 (5.0 ml) - 0.001 M sodium edetate solution pH 8.0 (10.0 ml) - water (up to 100.0 ml).
Flow rate: 1.0 ml/min; detection: UV at 254 nm; temperature: 60^0C.
1 = 4-epi-oxytetracycline, 2 = oxytetracycline, 3 = 2-acetyl-2-decarboxamido-oxytetracycline, 4 = tetracycline, 5 = a-apo- oxytetracyclinc, 6 = anhydro-oxytetracycline, 7 = b-apooxytetracycline, 8 = unknown.

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