Ars Separatoria Acta 8 (2011) 39-49

ARS SEPARATORIA ACTA
www.arsseparatoriaacta.com

DETERMINATION OF TRACE QUANTITIES OF DUTASTERIDE IN WATER AND WASTEWATER BY SOLID PHASE EXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Agnieszka WASILEWSKA[1], Marta MADAJCZYK[2],
Arkadiusz SZYMAŃSKI[2*], Włodzimierz URBANIAK[1,2]

[1]Faculty of Chemical Technology and Engineering,
University of Technology and Life Sciences, Seminaryjna 3, 85-326 Bydgoszcz
[2]Faculty of Chemistry, Adam Mickiewicz University,
Grunwaldzka 6, 60-780 Poznań, Poland
e-mail: arekszym@amu.edu.pl

ABSTRACT

Steroid compounds are a kind of pollutants which are increasingly more frequently found in environmental samples. Due to the ever more increasing level of such pollutants and their biological activity, they are of interest to analysts. Our study involved the development of a simple and fast method for the assay of dutasteride in treated industrial waste water using high-performance liquid chromatography. As the steroid concentration in water is much lower than the limit of detection of the UV detector used, the compound assayed in the test water was pre-concentrated using solid-phase extraction. Our procedure has high accuracy and a precision of 2.6%, and the recovery of the analyte from water is 78.6%. The procedure enables the assay of dutasteride at a level of 2 μg/L in water.

Keywords: dutasteride, emerging pollutants, solid-phase extraction (SPE), liquid chromatography (HPLC)

INTRODUCTION

It is one of the essential duties of environmental chemistry to develop methods for the detection and assay of biologically active in the natural environment, particularly in water. The presence of such compounds results from

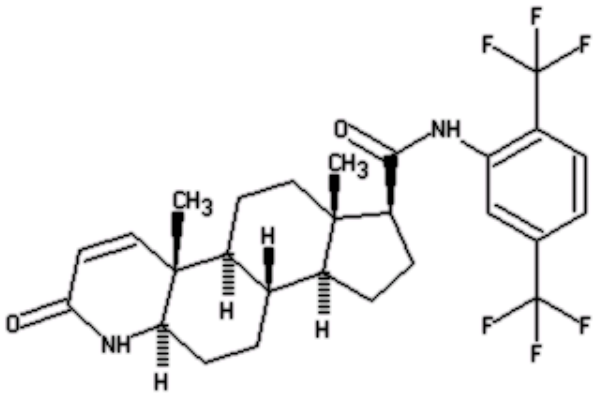
* Corresponding author

drug manufacturing, lack of proper disposal of formulations past their expiry date and excretion of drug residues and metabolites by living organisms [1]. Such compounds include, for example, steroids and hormones, surfactants, and drug residues. They are of an increasingly high interest and concern to analysts involved in environmental monitoring. First of all, this is due to the increasing level of such compounds in the natural environment and, secondly, due to their biological activity noted above [2]. Compounds of particular concern include pharmaceutical residues: antibiotics [3-5] and sulphonamides [6], because of issues such as the emergence of new bacterial strains which are resistant to drug groups, and Endocrine Disrupting Compounds, EDCs [7, 8]. The latter group includes, for example, alkylphenol ethoxylates and steroid compounds.

The production and use of steroid drugs increases the risk of exceeding acceptable levels in wastewater and also in potable water. This may be dangerous to human health and the environment. They are usually present in trace amounts; thus their monitoring is extremely difficult. According to industrial wastewater purity standards, steroid concentrations are limited to 1 to 2 μg/L.

Steroid compounds in industrial sewage usually originate from the washing of steroid processing systems, for example, in the pharmaceutical industry (washing water). Due to their very low solubility in water, steroids are used as solutions in special solvents, most often mono and diglyceride of medium chain fatty acids (mainly caprylic and capric) hereinafter referred to as oil solvents. Alkaline washing agents containing non-ionic surfactants, hereinafter referred to as detergents, are used, for washing the systems. The resulting sewage is then pre-treated through the oil layer separation and removal.

Steroids can be analysed and assayed using chromatographic methods such as gas chromatography [9] and high-performance liquid chromatography [10, 11]. The limit of quantification of HPLC procedures using direct sample injection is too high to detect very low steroid concentrations in water. Therefore, pre-concentration of the analytes present in the samples before their determination is required.



Dutasteride

It was an objective of our study to develop a simple, rapid and precise method for the assay of steroids in treated industrial waste water. The method is based on the preliminary extraction and concentration of dutasteride by solid-phase extraction and determinations by high-performance liquid chromatography with a UV detector.

EXPERIMENTAL

Apparatus

Chromatographic separation was performed using the following hardware: HP 1050 (Hewlett Packard) liquid chromatograph with a UV detector with a variable wavelength of 190 to 660 nm, LiChrospher 100 column, RP-18 endcapped 5 μm, 250 x 4 mm (Merck) and Rheodyne 7025 injection valve with a 25 μL sample loop. Methanol/water = 90/10% was the mobile phase.

Water with high purity, used for the preparation of the mobile phases and solutions, was obtained from Milli-Q (Millipore).

UV spectra show that the dutasteride absorption maxima are at 210, 245 and 280 nm. Due to the content of methanol in the mobile phase, elution could be carried out at the latter two wavelengths. As the intensity of steroid chromatographic peaks was similar for 245 and 280 nm, the 280 nm value was selected, having a lower baseline noise.

A Bakerbond spe vacuum manifold was used for the elution of SPE columns, Baker spe 12G system.

N 810 Laboport laboratory vacuum pump (KNF Neurberger).

Chemicals and materials

Methanol for HPLC was purchased from POCh (Gliwice, Poland). Dutasteride was purchased from AK Scientific Inc. (USA). The samples were subjected to a pre-concentration and clean-up step by solid phase extraction using 3 mL ODS cartridges (500 mg) (Bakerbond). Glyceryl Caprylate / Caprate from Abitec (Capmul MCM) was used as an oil solvent. A cleaning product from Ecolab (COSA CIP 90) was used as a detergent.

Dutasteride stock solution

The standard solution of dutasteride was prepared by accurately weighing 10 mg of the drug and was diluted in 100 mL volumetric flask with methanol to produce a stock solution of 100 μg/mL. The stock solution was used to prepare further dilutions of the standard solution.

The pre-treated sewage may still contain significant quantities of oil and detergent, compared with steroids. Therefore, it had to be verified then, whether their presence influenced the course of analysis at the stage of separation and concentration of the analyte using the SPE method or at the stage of determina-

tion using the HPLC method. Taking into consideration the typical sewage washing and pre-treatment procedures, water saturated with oil solvent and detergent was used. The solution was obtained by mixing equal quantities of 1% detergent solution and 1% water suspension of oil, and then removing the separating oil layer.

Solid Phase Extraction

Bakerbond ODS column conditioning. 5 mL of methanol, 5 mL of methanol/deionised water (1:1) solution and 5 mL of deionised water were successively passed through the column with the SiO_2-C_{18} bed so as to prevent the silica bed from drying.

Sample application. The prepared test steroid solution in water was filtered gravitationally through the column at a rate of 1-3 mL/min. The sorbent was washed with 2 mL of deionised water and vacuum-filtered with air for 20 min.

Elution. The analyte was eluted with 2 mL methanol volume, evaporated to 0.5 mL and analysed chromatographically.

Determination of dead time. In order to determine the dead time value for the chromatographic system used, aqueous sodium nitrite was injected. Injections were repeated 6 times, and average dead time was determined based on the resulting retention times of 1.7 min.

RESULTS AND DISCUSSION

Repeatability of steroid retention. The steroid (20 μg/mL concentration) was injected 10 times onto the column. Average retention time was 3.200 min. Very good repeatability with high precision, indicated by the low relative standard deviation value, was noted.

$$t_R = 3.200 \pm 0.004 \text{ min} \qquad RSD = 0.125\%$$

Calibration curve. A linearity of the relationship between the steroid peak area was determined based on a calibration curve with the general formula $y = ax + b$. The calibration curve was determined based on chromatographic analysis results for water samples saturated with oil solvent (10 g/L) and detergent (7.6 g/L) and spiked with the test steroid. The solutions contained 5, 10, 15, 20 and 25 μg of the steroid, respectively, per 100 mL of water. The solutions were filtered according to the above procedure through ODS-filled columns and analysed chromatographically. Three replicates were analysed for each solution.

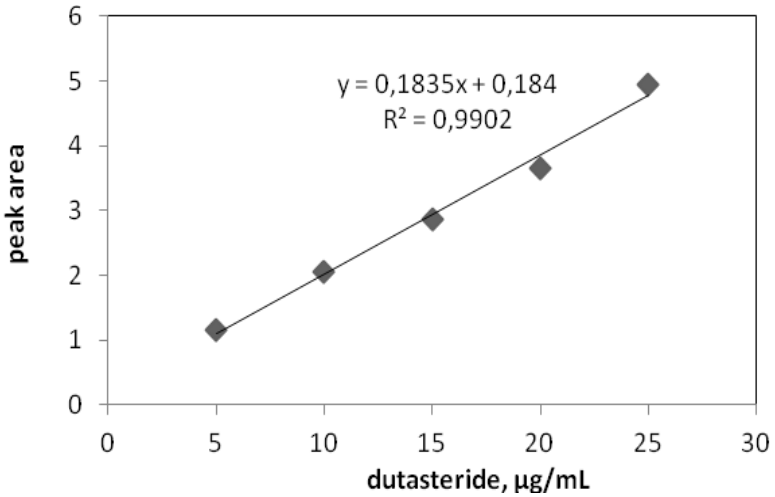


Fig. 1. Calibration curve for dutasteride.

Curve parameters: the slope (a), constant (b), and square of linear correlation values are shown in the figure. A high linear correlation was achieved in the concentration range tested.

Recovery determination. To determine the analyte recovery from water using our method, aqueous solutions (2.5 L containing 5 µg of the steroid, concentration in water: 2 µg/L) were passed through the SPE columns with ODS. The aqueous solutions were passed owing to the difference of levels between the solution filtered and the column. The steroid solubility in pure water is very low (2.1 µg/L). The steroid extracts were applied onto the chromatographic column and analysed. Each injection was further repeated twice. The following chromatographic peak areas were obtained: 0.78; 0.80; 0.76; 0.77; 0.77 and 0.74. The measurement precision, as expressed by RSD, was 2.60%. The average peak area of the steroid assayed was 0.77 ± 0.02 and the average steroid standard peak area (10 µg/mL concentration) for three injections was 0.98. Based on the results, the recovery of the method for the steroid concentration and assay was determined to be 78.6%.

Limit of detection. The limit of detection was determined for the blank sample (no steroid), prepared according to the procedure specified for the determination of recovery. Concentration was performed twice by injecting each extract 3 times. The limit of detection, LOD, was calculated for a signal 3-times as high as the noise level.

$$LOD = 4.8\ \mu g/mL$$

Chromatographic analysis. The mobile phase was a 90:10 mixture of methanol and water. Dutasteride retention time was 3.2 min (Fig. 2). At the selected eluent composition, the chromatographic peaks of the oil or the detergent had

shorter retention times as seen in the chromatograms below (Fig. 3 and 5). Figs. 4, 6 and 7 show chromatograms of the separation of a mixture containing water saturated with the oil and the detergent with dutasteride added. The chromatograms confirm that it is possible to determine the analyte when the additives, found in industrial wastewater when preparing dutasteride formulations, are present.

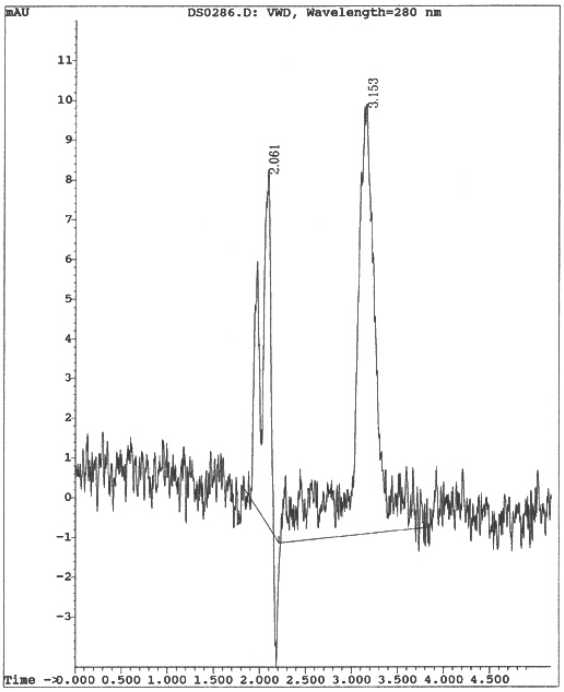


Fig. 2. Chromatogram of dutasteride standard, 20 µg/mL.

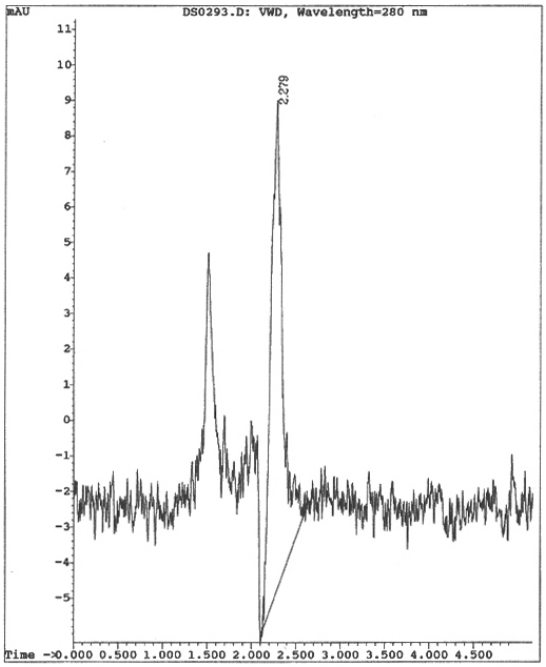


Fig. 3. Chromatogram of water with oil solvent.

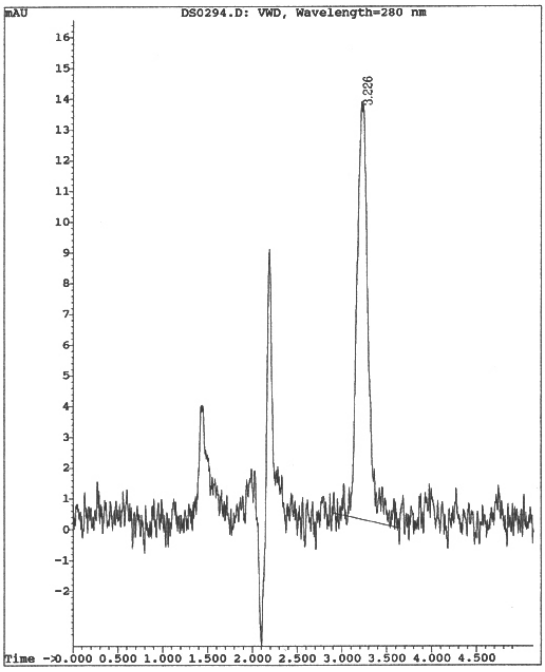


Fig. 4. Chromatogram of water with oil solvent with dutasteride added, 10 µg/mL.

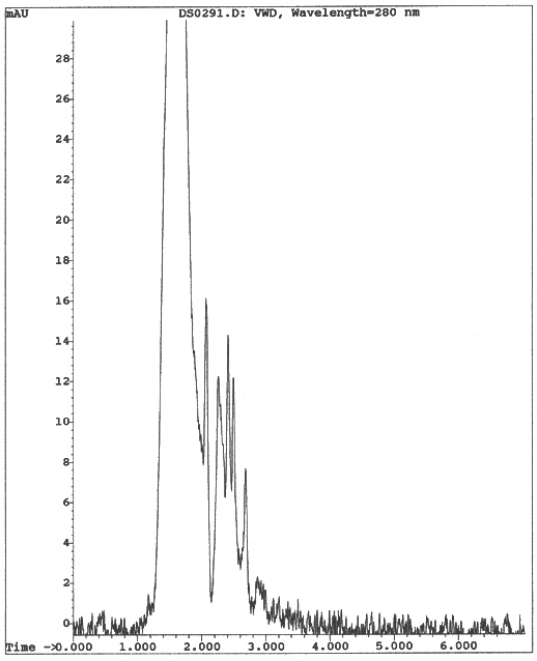


Fig. 5. Chromatogram of water with oil solvent and detergent.

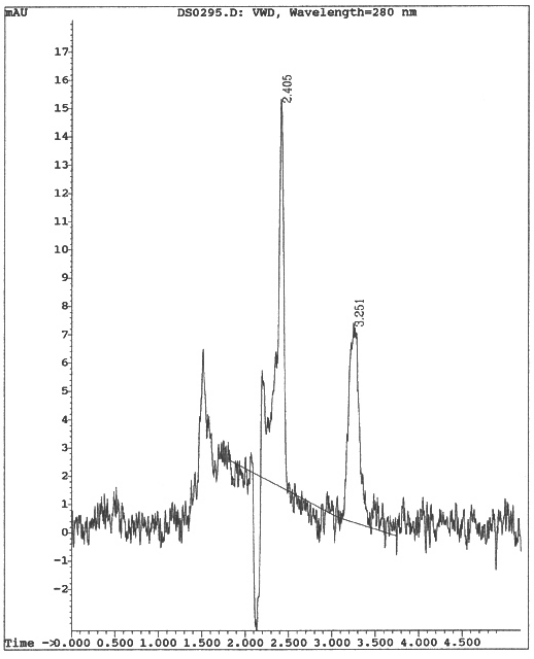


Fig. 6. Chromatogram of water with oil solvent and detergent with dutasteride standard added, 10 μg/mL.

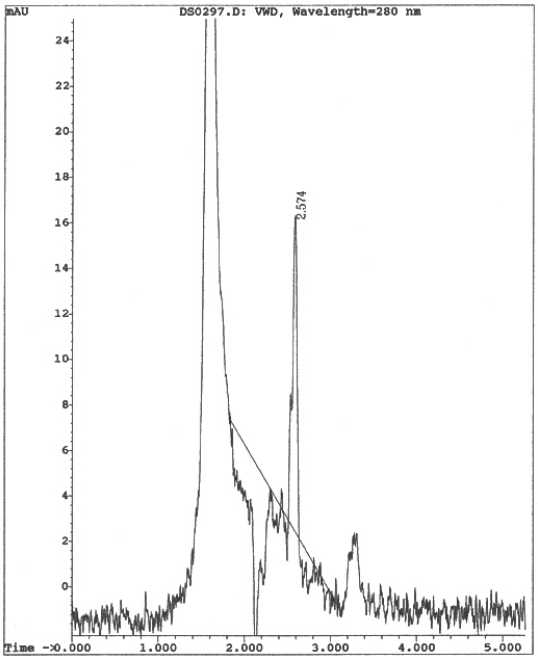
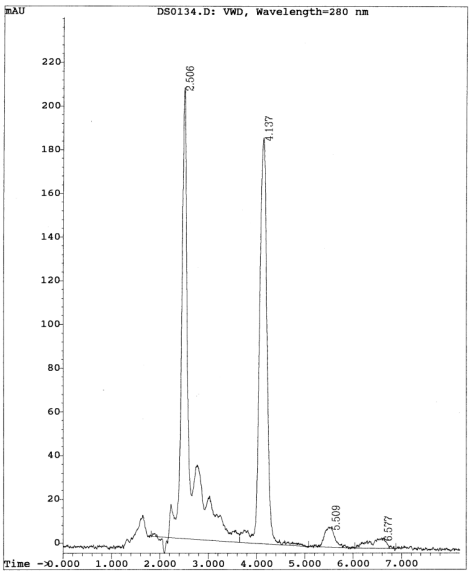
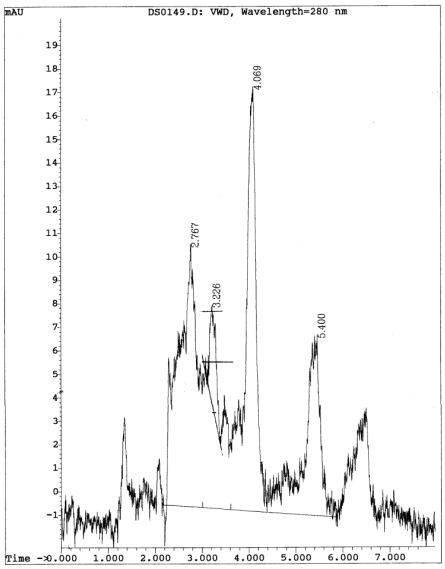


Fig. 7. Chromatogram of water with oil solvent and detergent with dutasteride standard added, 5 μg/mL.

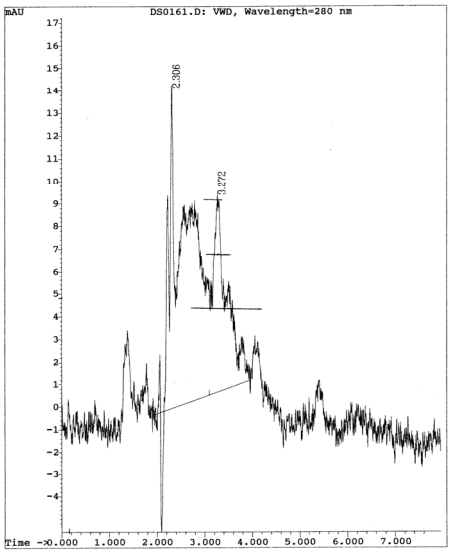
Effect of other factors on steroid determination. In Figure 8, three chromatograms are compared, recorded as discussed for determination of recovery. Differences between the chromatograms result from the fact that different materials are used for concentrating the 2.5 L water samples with the analyte. Chromatogram 1 shows a sample in which a polyethylene hose for passing the aqueous steroid solution onto the SPE column and a plastic container for water storage were used. Chromatogram 2 shows a sample in which the polyethylene hose was replaced by a Teflon one while chromatogram 3 corresponds to the elimination of the plastic container as well. In addition, the glass bottle inlet was secured by a column with activated carbon to prevent any absorption of pollutants from air.



Chromatogram 1



Chromatogram 2



Chromatogram 3

CONCLUSION

The steroid chromatographic peaks obtained in the chromatographic conditions used were appropriate for quantitative analysis interpretation. Within the dead time and below the analyte retention time, peaks from or due to the oil and the detergent present in the water occurred. The peaks, however, do not interfere with dutasteride determination. Our studies confirm the suitability of the

method for the assay of the steroid in water disposed of as sewage. The method has high accuracy and precision. Using the procedure, the steroid at a level of 2 μg/L can be assayed in water.

ACKNOWLEDGEMENTS

Financial support from Ministry of Science and Higher Education (Poland) - Grant N204/0253/38 and European Funds - Grant No UDA-POIG.01.03.02-00-038/09-00.

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