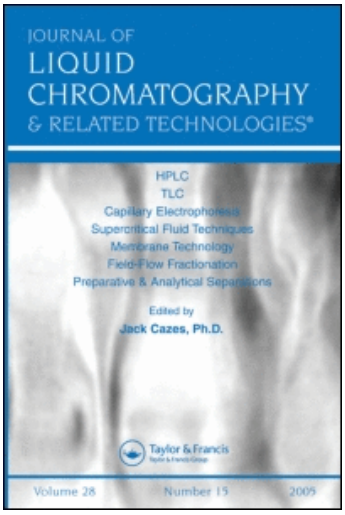

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Aztreonam by liquid Chromatography with UV and Amperometric Detection

Xinjian Huang[a]; W. Th. Kok[a]; H. Fabre[b]
[a] Laboratory for Analytical Chemistry University of Amsterdam Nieuwe Achtergracht, Amsterdam, The Netherlands [b] Laboratoire de Chimie Analytique, Faculté de Pharmacie, Montpellier Cedex, France

To cite this Article Huang, Xinjian , Kok, W. Th. and Fabre, H.(1991) 'Determination of Aztreonam by liquid Chromatography with UV and Amperometric Detection', Journal of Liquid Chromatography & Related Technologies, 14: 14, 2721 — 2733
To link to this Article: DOI: 10.1080/01483919108049351
URL: http://dx.doi.org/10.1080/01483919108049351

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(14), 2721-2733 (1991)

DETERMINATION OF AZTREONAM BY LIQUID CHROMATOGRAPHY WITH UV AND AMPEROMETRIC DETECTION

XINJIAN HUANG[1], W. TH. KOK[1]*,
AND H. FABRE[2]
[1]*Laboratory for Analytical Chemistry*
University of Amsterdam
Nieuwe Achtergracht 166
1018 WV Amsterdam, The Netherlands
[2]*Laboratoire de Chimie Analytique*
Faculté de Pharmacie
34060 Montpellier Cedex, France

ABSTRACT

A comparative study of UV and amperometric detection of aztreonam after HPLC separation is presented. At pH 2.0 and a detection potential of +1.15 V (vs. Ag/AgCl), the detection limits with amperometric detection are about two times higher (3-5 ng) than those obtained with UV detection (1-3 ng) for aztreonam and its main decomposition products, the E-isomer and open-ring aztreonam. With the advantage of specificity for the aminothiazole group of the aztreonam molecule, amperometry can be used as an alternative or complementary mode to UV detection for the determination of aztreonam in injectable formulations and in human serum.

INTRODUCTION

Aztreonam is a new synthetic monocyclic beta-lactam antibiotic in the recently defined family of monobactam antimicrobial compounds, which is highly active against a broad range of aerobic gram-negative bacteria (1). The advantages of high-performance liquid chromatography (HPLC), compared with microbiological assays, namely its selectivity and speed, have been widely reported. Several HPLC methods, all using UV-absorption as detection mode, have been proposed to determine the stability of the drug in bulk and dosage form and to monitor the antibiotic in biological fluids (2-9).

Aztreonam and its two main degradation products (10), the E-isomer and open-ring aztreonam (Figure 1), have an aminothiazole group in the side chain of the β-lactam structure. It was shown previously that cephalosporins with the same functional group could be detected successfully by direct and indirect amperometry (11,12). In the present work the possibility of using amperometric detection as an alternative or complementary mode to UV-absorption detection has been investigated. The method has been validated for the monitoring of aztreonam in injectable formulations and in human serum.

EXPERIMENTAL

Apparatus

The chromatographic equipment consisted of a Gynkotek (Germering, FRG) Model 300C pump with an extra home-made pulse-damper, a

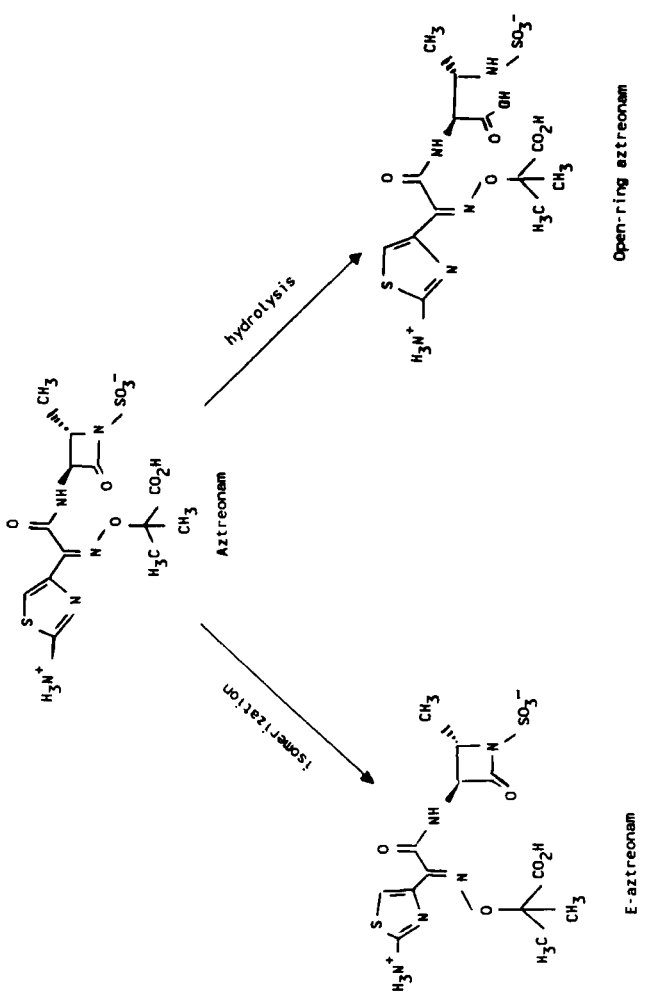

FIGURE 1. Aztreonam and degradation products studied.

Rheodyne injection valve with a 30 μl loop, a 150 * 4.6 mm i.d. column, slurry packed with Hypersil ODS (5 μm), a Kratos (Ramsey, NJ, USA) Spectroflow 757 variable-wavelength UV detector and downstream in series a Spark (Emmen, The Netherlands) AMOR amperometric detector with an analogue potentiostat-amplifier. The working electrode was of glassy carbon and the reference electrode was an in situ Ag/AgCl electrode. The working electrode was cleaned every day before the experiments by wiping it with methanol. The time constants of the potentiostat-amplifier and UV detector were set at 1 s.

An Eppendorf 5414S centrifuge with 15000 R.P.M. was used for the deproteination of serum samples.

All experiments were conducted at room temperature (20±2°C).

Chemicals and solutions

Aztreonam (SQ 26776, batch#AZ037), open-ring aztreonam (SQ26992, lot#NN006) and E-isomer of aztreonam (SQ28429, lot#NN004) were obtained from the Squibb Institute for Medical Research, USA. Azactam powder, containing aztreonam and L-arginine, was obtained from Bristol Laboratories (Paris, France). Acetonitrile was HPLC grade. All other chemicals were of analytical-reagent grade.

Aqueous stock solution (0.2 g l^{-1}) of aztreonam was prepared with water and daily suitably diluted with water to the required concentration. For stability studies, aztreonam solutions with and without arginine were divided into two parts and kept at room temperature and in a refrigerator, respectively, and diluted to 10 μg ml^{-1} in water before injection.

Mobile phases for reversed-phase liquid chromatography were prepared by mixing methanol with a 0.1 mol l^{-1} phosphate buffer solution adjusted to different pH values. Potassium chloride (0.01 mol l^{-1}) was added to the mobile phases for the stabilisation of the reference electrode. The mobile phases were de-aerated with helium before use.

Sample preparation

For recovery studies pooled human serum samples were spiked with 37 μg ml^{-1} and 8 μg ml^{-1} of aztreonam, respectively. Deproteination was carried out by mixing an aliquot (0.5 ml) of serum with an equal volume of acetonitrile in a capped vortex vial, mixing thoroughly for 10 s and centrifuging for 2 min at 15000 R.P.M. Then an aliquot (0.5 ml) of the supernatant was diluted 1+1 with water before injection.

RESULTS AND DISCUSSION

Electrochemical characteristics

The electrochemical oxidation of aztreonam was investigated at pH 2.0, 4.0 and 7.0. Figure 2 shows the hydrodynamic voltammograms. The halfwave potential shifts only slightly to lower values as the pH is increased. Real current plateaus were not found for any pH value within the voltage range studied. The shape of the voltammetric waves is more reversible at lower pH. In experiments with cyclic voltammetry the oxidation of aztreonam was found to be irreversible under similar conditions.

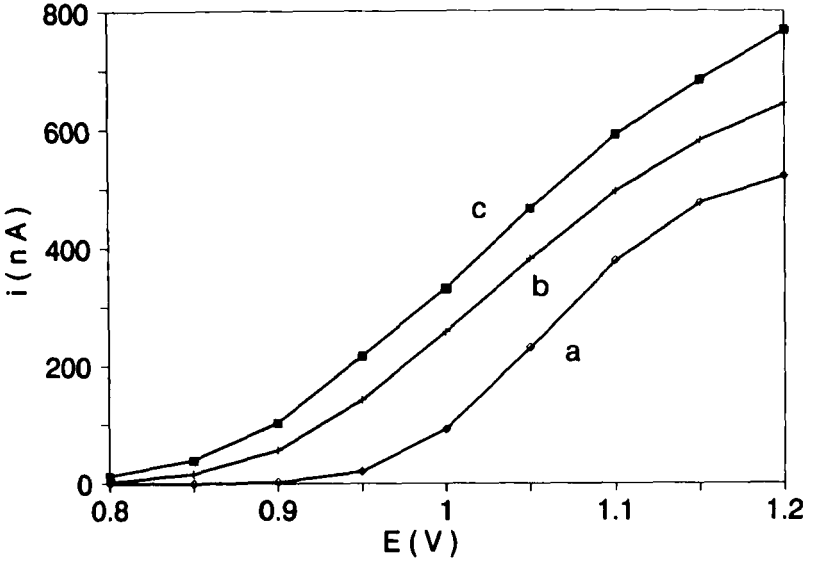

FIGURE 2. Hydrodynamic voltammograms of aztreonam. Mobile phase: 0.1 mol l^{-1} potassium phosphate buffer, 0.01 mol l^{-1} potassium chloride, 20% (v/v) methanol. (a) pH = 2, (b) pH = 4, (c) pH = 7.

A high background current and noise was observed at pH 7.0 at potentials more positive than +1 V. Optimal signal-to-noise ratios were found at a pH value of 2.0 and a detection potential of +1.15 V (vs. Ag/AgCl). The lower maximum peak-heights observed at low pH can be attributed to an increase of the retention time and peak dilution. With mobile phases containing 20% (v/v) methanol, with pH-values of 2, 4 and 7, the retention times of aztreonam were 6.3, 3.5 and 2.3 min, respectively.

Indirect amperometric detection, with on-line generated bromine as oxidizing reagent, was also investigated. The rate of the reaction of aztreonam with bromine was low compared to that of

other sulphur-containing compounds (e.g., cephalosporins [12]) which resulted in signal-to-noise ratios inferior to those obtained with direct amperometry. Because of the lack of sensitivity no further investigation was carried out with indirect amperometric detection.

Linearity, sensitivity and precision

The two main degradation products of aztreonam, the E-isomer and the open-ring species, possess the same aminothiazole group in their side-chain as aztreonam and can be detected electrochemically. Standard solutions of aztreonam and its two degradation products were injected, using a mobile phase of pH 2.0 with 20% (v/v) methanol. The retention times were 6.6 min for aztreonam, 3.9 min for open-ring aztreonam and 13.0 min for the E-isomer. For calibration, peak heights at 268 nm (UV) and +1.15 V (amperometry) were used. With UV detection calibration plots for the three compounds were linear ($r \geq 0.998$) and passed through the origin within the range 30 - 4500 ng injected. For amperometric detection, the calibration graph was linear ($r=0.9997$) within a 30 - 900 ng range. Above this linear range the sensitivity decreased gradually, probably by partial poisoning of the electrode surface by the oxidation products. Table 1 summarizes the sensitivities and limits of detection with both detectors. With UV lower detection limits could be obtained.

The precision was assessed by six successive injections of aztreonam (300 ng injected). The coefficients of variation were below 2.0% for both detection modes.

TABLE 1

Sensitivities and Limits of Detection with UV and Amperometric Detection

Compound	UV detection [268 nm]		Amperometry [+1.15 V]	
	sensitivity [mAU ng^{-1}]	LOD[a] [ng]	sensitivity [nA ng^{-1}]	LOD [ng]
Aztreonam	0.081	1	0.975	3
Open-ring	0.125	1	0.802	4
E-isomer	0.033	3	0.635	5

[a]: Limit of detection, signal to noise ratio = 3

Application to pharmaceutical formulations

The method was applied to the determination of aztreonam in the commercial form formulated with arginine, Azactam. Trace contaminants were detected with UV-absorbance and amperometric detection, indicating that in these contaminants the aminothiazole group is present in their structure. Under the conditions used, arginine was not detected.

The assay of aztreonam in the formulation yielded a weight-fraction of 57.7 ± 1.9 % with UV detection and 57.5 ± 1.3 % (n=3) with amperometric detection, respectively. The accuracy of this result was assessed by comparison with the USP procedure as stated in the monograph "sterile aztreonam" (10). The results obtained with the USP method (56.2 ± 1.5 %) compare well with those of the proposed procedure.

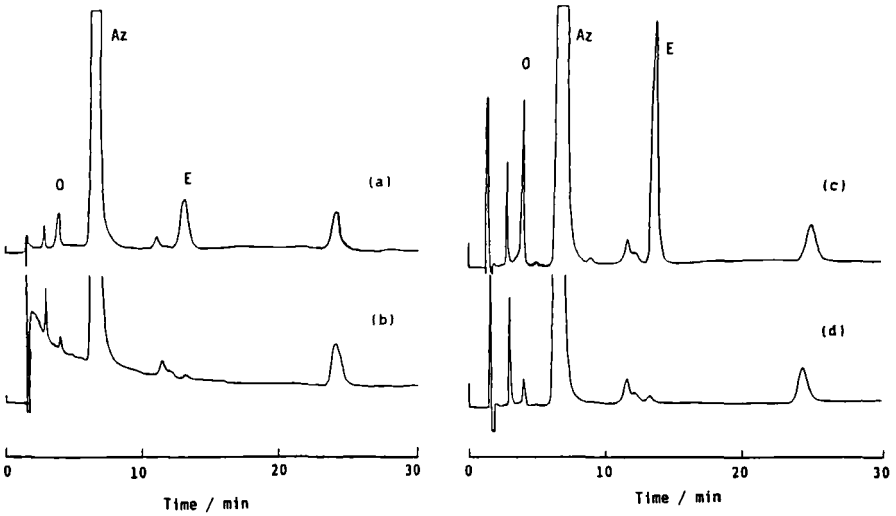

FIGURE 3. Stability of an aztreonam solution (200 μg ml^{-1}). (a) and (c): solution after 7 days storage at room temperature; (b) and (d): the same solution freshly prepared. (a) and (b): amperometric detection; (c) and (d): UV detection. Aztreonam (Az), open-ring aztreonam (O) and the E-isomer (E). For experimental conditions see text.

With UV and amperometric detection the stability of aztreonam and Azactam solutions can be monitored. A decrease of the concentration of a 200 mg l^{-1} solution of about 4% and 10% was observed after 7 days storage at 4 °C and room temperature, respectively. The presence of arginine stabilizes the solutions since only a 5% decrease was observed after 7 days of storage of an Azactam solution at room temperature. The two major decomposition products formed are the E-isomer and open-ring aztreonam (see Figure 3). The formation of the E-isomer is more important in aztreonam solution (acidic) than in Azactam solution.

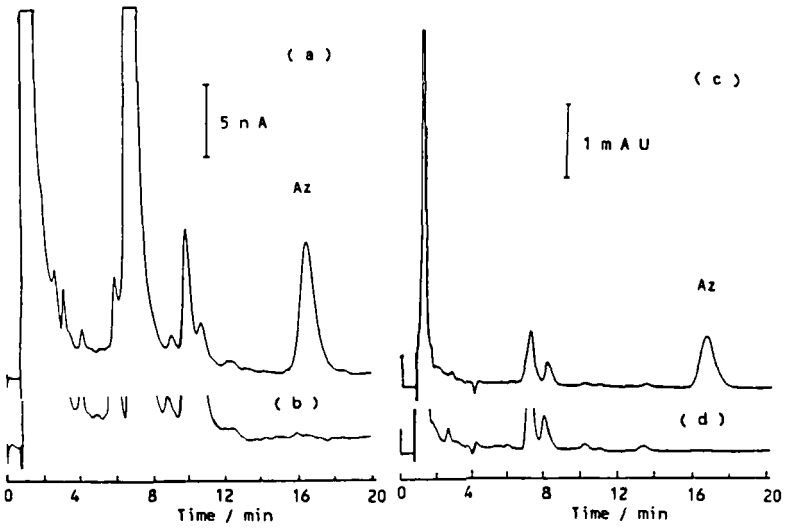

FIGURE 4. Determination of aztreonam in human serum.
(a) and (c): serum spiked with 8 $\mu g\ ml^{-1}$ of aztreonam (Az);
(b) and (d): blank serum. (a) and (b): amperometric detection;
(c) and (d): UV detection. For experimental conditions see text.

This is in agreement with the observations reported by Florey (13), stating that in acidic medium aztreonam is partially converted to its isomer.

Application to serum samples

The method was applied to the determination of aztreonam in serum. For most applications to biological samples, the highest sensitivity is not required. Therefore, to prolong the life time of the column, the samples were then diluted after pretreatment. For the analysis of (spiked) serum samples, a better separation from the matrix components could be obtained using a mobile phase

TABLE 2

Recovery of Aztreonam from Spiked Human Serum.

Concentration[a] [$\mu g\ ml^{-1}$]	Recovery [%]	
	UV detection	Amperometry
8	98.8 ± 1.2 (n=3)	97.3 ± 1.3 (n=3)
37	95.7 ± 6.0 (n=4)	95.2 ± 5.6 (n=4)

[a]: before dilution
n: number of extracted samples

containing 0.1 M phosphate buffer (pH 3.0) and methanol (91+9). At a flow rate of 1.8 ml min^{-1}, the retention time of aztreonam was 16.5 min. Chromatograms of a blank serum sample and a spiked serum solution are shown in Fig.4. The selectivity of UV detection is superior to that of amperometric detection. Table 2 gives the results of recovery studies for different concentrations of aztreonam in serum. The recovery is approximately quantitative in the concentration range studied. Statistical analysis of the experimental results showed that the pretreatment is the major source of the variance. The limit of determination (SNR = 10) of aztreonam in serum is 1 $\mu g\ ml^{-1}$ with UV detection and 2 $\mu g\ ml^{-1}$ with amperometric detection.

CONCLUSION

Electrochemical detection can be used for the determination of aztreonam in pharmaceutical formulations as well as in biological

samples. UV detection is slightly superior to amperometric detection in respect to sensitivity as well as selectivity. However, amperometric detection can be used as a complementary mode to UV detection to confirm the identity of peaks, since amperometric detection is related to the aminothiazole group in the side chain of the molecule.

REFERENCES

1. Rouan, M.C., Antibiotic Monitoring in Body Fluids, J.Chromatogr., 340, 379., 1985.

2. Swabb, E.A., Leitz, M.A., Pilkiewicz F.G. and Sugerman, A.A., Pharmacokinetics of the Monobactam SQ 26776 after Single Intravenous Doses in Healthy Subjects, J.Antimicrob. Chemother., 8, 131, 1981.

3. Pilkiewicz, F.G., Remsburg, B.J., Fisher S.M. and Sykes, R.B., High-pressure Liquid Chromatographic Analysis of Aztreonam in Sera and Urine, Antimicrob. Agents Chemother., 23, 852, 1983.

4. James M.J. and Riley, C.M., Stability of Intravenous Admixtures of Aztreonam and Clindamycin Phosphate, Am.J.Hosp.Pharm., 42, 1095, 1985.

5. Adamovics J. and Unger S., Preparative Liquid Chromatography of Pharmaceuticals using Silica Gel with Aqueous Eluents, J.Liquid Chrom., 9, 141, 1986.

6. Meulemans, A., Mohler, J., Vittecoq, D., Haroche, G., Rosset, M.A., Vulpillat M. and Modai, J., High-performance Liquid Chromatographic Analysis of Azthreonam in Tissues and Body Fluids, J.Chromatogr., 377, 466, 1986.

7. Marble, D.A., Bosso J.A. and Townsend, R.J., Stability of Clindamycin Phosphate with Aztreonam, Ceftazidime Sodium, Ceftriaxone Sodium or Piperacillin Sodium in Two Intravenous Solutions, Am.J.Hosp.Pharm., 43, 1732, 1986.

8. Jehl, F., Birckel P. and Monteil, H., Hospital Routine Analysis of Penicillins, Third-Generation Cephalosporins and Aztreonam by Conventional and High-speed High-performance Liquid Chromatography, J.Chromatogr., 413, 109, 1987.

9. Wahbi, A.A.M., Abounassif, M.A., Gad-Kariem, E.R.A., Ibrahim, M.E. and Aboul-Enein, H.Y., Determination of Aztreonam and L-Arginine Combination in Parental Formulations, J.Ass.Off.Anal.Chem., 71, 31, 1988.

10. United States Pharmacopoeia, USP XXII, Mark Publishing Company, Easton, 1989, 129-131.

11. Fabre, H., Blanchin M.D. and Kok, W.Th., Liquid Chromatography with Amperometric Detection for the Determination of Cephalosporins in Biological Fluids, Analyst, 113, 651, 1988.

12. Fabre H. and Kok, W.Th., Determination of Cephalosporins and Decomposition Products by Liquid Chromatography with Indirect Electrochemical Detection, Anal. Chem., 60, 136, 1988.

13. Florey, K., Analytical Profile of Drugs, Vol. 17, Academic Press, London, 1988, pp. 1-39.