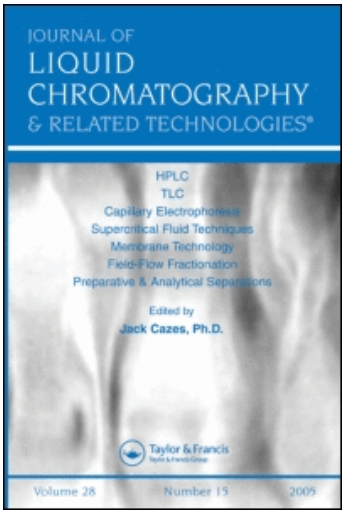

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THE "ON LINE" DETERMINATION OF CEFSULODIN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN CSF AND PLASMA OF RATS

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ABSTRACT

After an intraveinous injection of 20 $mg.kg^{-1}$ of cefsulodin in anaesthetized rats, the cerebrospinal fluid (CSF) and plasma drug levels were determined on line by HPLC. The CSF was withdrawn from the IIIe venticle at a flow rate of 1 µl.minute and pushed with a peristaltic pump, via a teflon tube into a "micro vial" inside the WISP vial. At the end of fiveteen minutes, about 15 µl of CSF was collected and a small volume (7 µl) was injected by the WISP into the column, the excess of CSF was discarded by another teflon tube inside the "micro-vial". After the injection, the continous flow of CSF filled the "micro vial", an injection was running at fiveteen minutes and so on during three hours. Plasma samples were collected every thirty minutes, and after methanol extraction cefsulodin levels were determined by HPLC. Given the low drug levels found in the CSF, this method appeared very sensitive; it was a direct, rapid, automatic technique. Other biological molecules can be determined by this automatic dosage.

INTRODUCTION

The determination of cerebrospinal fluid concentration of antibiotics provides an index of diffusion through the blood brain barrier. This parameter is important in infections of the neurological sphere.

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In this study, we have tested a new semi-synthetic cephalosporin : cefsulodin in healthy rats. This drug is used against Pseudomonas aeruginosa infections in clinical practice. We had tested a new method to obtain cerebrospinal fluid from rats. A cannula was inserted in the IIIe venticle via a stereotaxic apparatus (1,2).

As described Mignot (3), we have used a continous aspiration from the third venticle. The collection on line of CSF allowed direct determination of drug levels by High Performance Liquid Chromatography (4). This paper describes an automatic method by a self acting sample and simultaneous determination. An "on line" kinetic study during three hours allowed us to follow "in vivo" the drug diffusion into CSF of rats.

MATERIALS AND METHODS

1) Animal preparation

Experiments were performed on Wistar rats (mean weight 340 g) anaesthetized with urethan (1.25 $g.k^{-1}$). A catheter was inserted in the carotid artery to allow injection of the antibiotic and to collect blood samples at fixed times.

The animals were positionned on a stereotaxic table and a cannula with its mandrel (1 mn external diameter) was placed in the IIIe venticle (6 mn under the dura, beyond the bregma). The mandrel was removed and replaced by a needle (0,5 mn external diameter). This needle was connected with a teflon tube to a peristaltic pump (Gilson). The CSF flow rate was 1 µl/minute and remained constant thoughout the experiment.

CSF was sampled every 15 minutes and plasma was collected every 30 minutes in heparined tubes for future analysis.

2) CSF sampling procedure

The continous CSF flow rate was achieved by a peristaltic pump (GILSON, France) and collected in a "micro vial". This "micro vial"

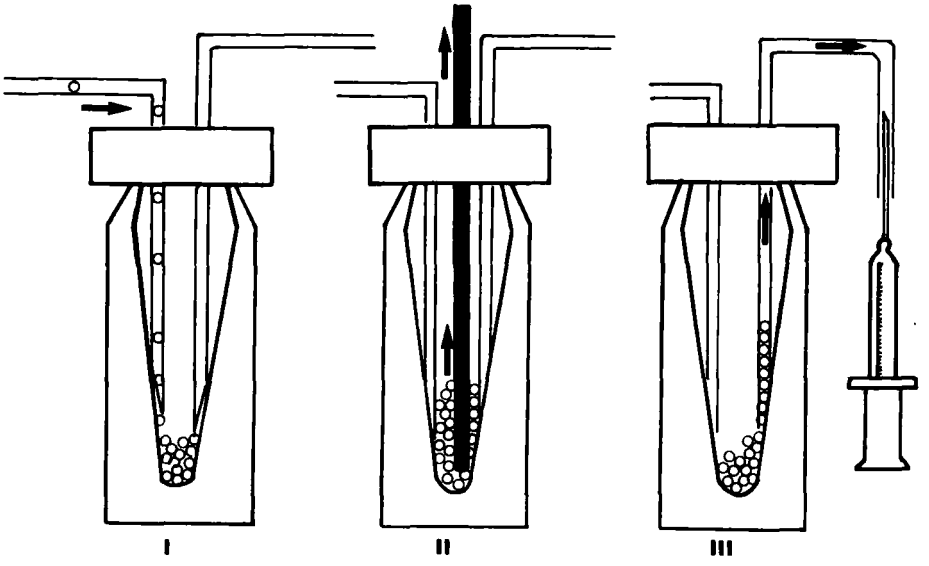

Figure 1. Schematic representation of the "micro vial".
The injection process was made in three times :

I : CSF withdrawn from rat, and filled the "micro vial".

II : at the end of 15 minutes the WISP needle, withdrawn 7 µl of CSF for injection into the HPLC column.

III : the excess of CSF was discarded by the seringe.

was in fact the "Limited Volume Insert" placed in the WISP vial (Waters, France). Two teflon tubes placed in the bottom. The first received the CSF from the pump; (figure 1 (I)). At the end of fiveteen minutes about 15 µl of CSF was collected; a small volume (7 µl) was sampled by the injection system of the WISP A 710 (Waters, France). The injection needle went into the "micro vial" and withdrawn 7 µl which then passed through the HPLC column (figure 1 (II)). The cycle then repeated it self every 15 minutes.

3) HPLC technique

a) Chromatographic equipment

Analysis was performed with a Waters Liquid Chromatograph (Waters Associates, St Quentin France) equipped with a WISP 710 A

injector, a 6000 A pump, a model 440 absorbance detector and a 10 mv recorder.

b) Solvents and standards

Fresly distilled deionised water was used : Methanol, acetronitrile were analytical grade (Merck, Darmstadt, Germany). Acetic Acid and ammonium acetate were ACS grade (Prolabo, France).

Cefsulodin reference standard vials were obtained from Cassenne (Paris, France).

c) Chromatographic conditions

The mobile phase was a mixture of acetonitrile, methanol ammonium acetate solution (0.02 $M.l^{-1}$) and acetic acid (v/v : 15/35/46/4). The eluent was ajusted to pH 4.1 by addition of acetic acid. The mobile phase was filtered through a 6 µm filter (Millipore, St Quentin, France) and degassing using ultrasonics.

The chromatographic separation was achieved using a µbondapak C18 column (Waters) with a flow rate at 1.5 $ml.min^{-1}$.

The absorbance detector was set a 280 mm with a sensitivity of 0.02 units full scall for the plasma and 0.005 units full scale for the CSF.

Two standard calibration curves were used. One curve for the CSF was made from a solution of cefsulodin (1 $mg.ml^{-1}$) diluted with distilled water to obtain concentrations ranging between 0.5 to 8 $\mu g.ml^{-1}$. The second curve was made from the same aqueous solution fo cefsulodin, diluted with plasma without drug to obtain concentrations from 1 to 100 $\mu g.ml^{-1}$.

From the arterial blood sampled in heparined small conical tubes 50 µl of plasma were added 50 µl pooled human plasma and 150 µl of methanol. The samples were mixed for ten minutes, then centrifuged at 2000 g for ten minutes. The supernatant was injected directly into the chromatograph.

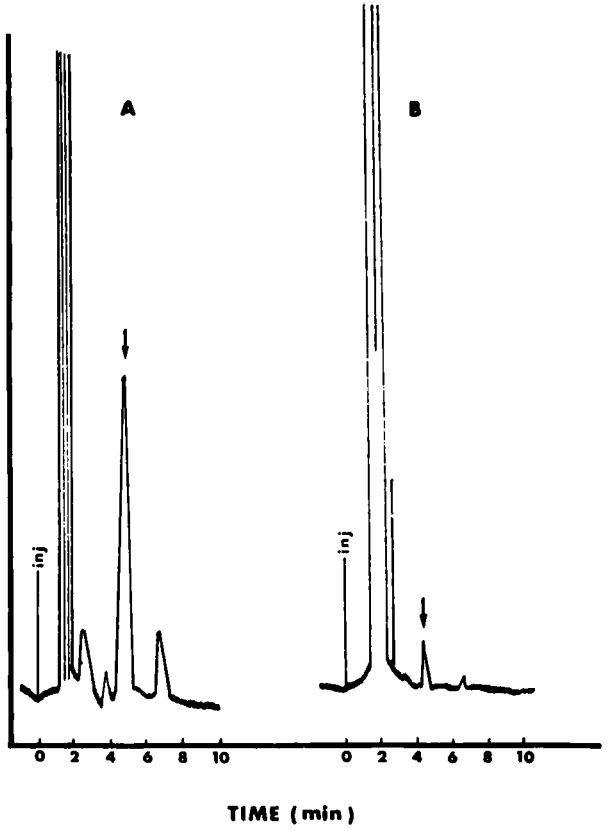

Figure 2. Chromatographic separation of cefsulodin in extracts of plasma (A) and cerebrospinal fluid (B).

RESULTS

The retention time of cefsulodin was 4.8 minutes under the described conditions : the chromatogram is shown in figure (2).

The chromatogram in figure (3) shows in continous determination of the penetration of cefsulodin into the CSF. The first chromatogram corresponded to CSF without drug.

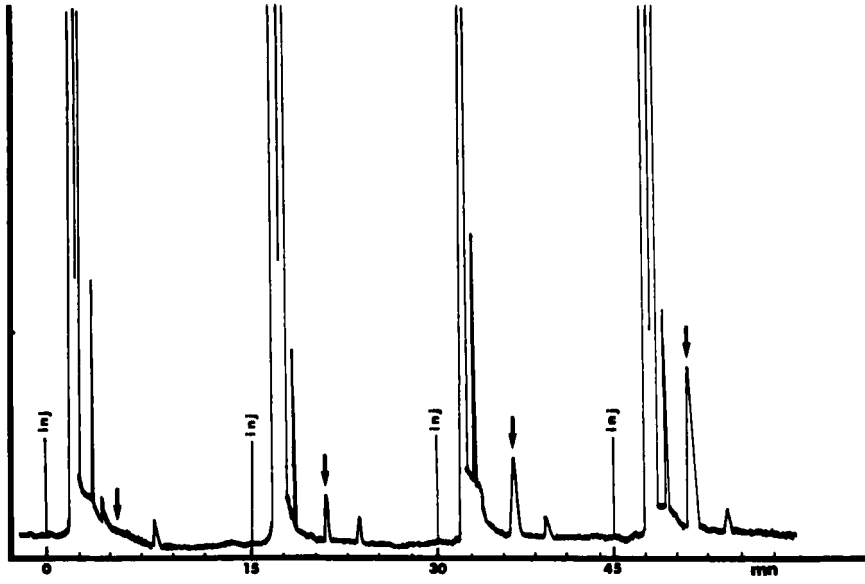

Figure 3. Chromatograms of "on line" determination of cefsulodin. The first one, at t = 0, corresponded at CSF without drug. The other chromatograms shown the increase of the drug with time.

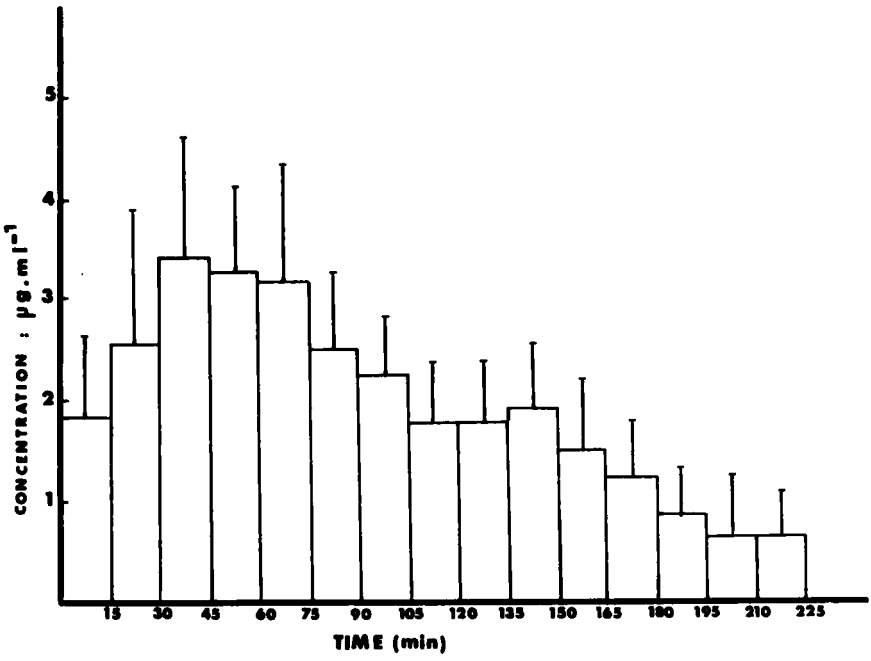

Figure 4. Diffusion of cefsulodin in the CSF of healthy rats (dose 20 mg/kg) Each space represented the average of five determinations.

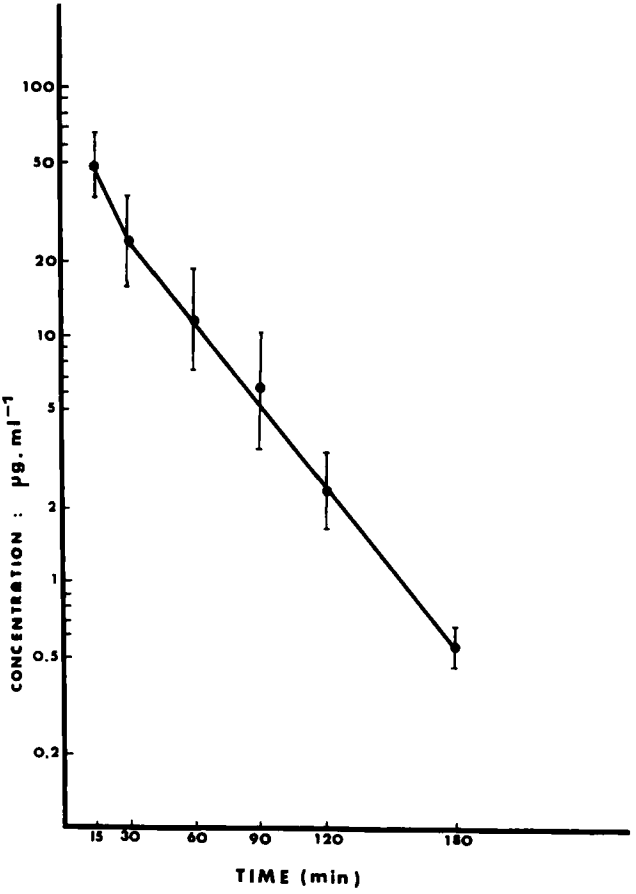

Figure 5. Plasmatic kinetic of cefsulodin.
Results plotted semilogarithmiccaly ; T 1/2 was at twenty five minutes : each point represented the average of five determinations.

The cefsulodin levels found in CSF are described in the figure (4). The peak concentration was observed at 30' (plateau at 3 $\mu g.ml^{-1}$) and remained unchanged until 75'. After that it decreased slowly reaching a level of 0.8 $\mu g/ml^{-1}$ about three and half hours.

Plasmatic concentrations are shown in figure (5). Results are plotted semi-logarithmically and are the mean concentration of five samples. Plasmatic levels decreased in a linear fashion reaching a levels of 0.5 $\mu g.ml^{-1}$ at 3 hours with a T 1/2 of twenty five minutes.

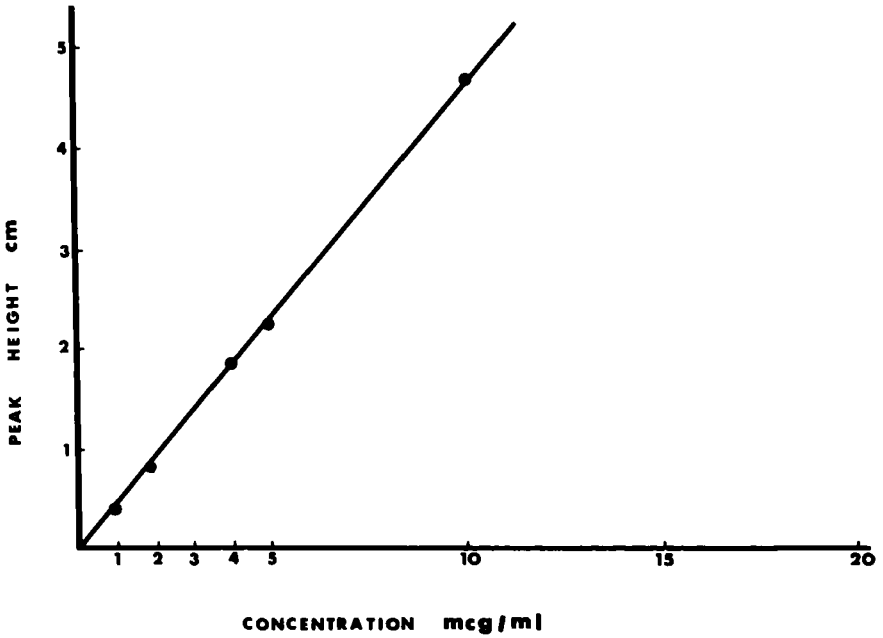

Figure 6. Standard curve cefsulodin determination in CSF using a 7 µl sample volume.
Chromatographic peak eight expressed as a function of concentration in CSF.

The calibration curve of cefsulodin in the CSF is illustrated in figure (6). As showed with an injection of 7 µl CSF, it was not possible to reach level < at 0.3 $g.ml^{-1}$ (peak heigh 0.3 cm). The detector was set a maximum of sensitivity, it was impossible to greater volumes of CSF. Considering that 15 µl was collected in the "micro vial" during 15 minutes. A minimum sample volume was required to cover the needle port during sample withdrawal; about 6 µl was needed to covert the needle port. The volume of 7 µl of CSF seems be the limit volume for injection. The limit of detection for plasma samples was set a 0.3 $\mu g.ml^{-1}$ under the present conditions. By slightly increasing the volume of injection (80 µl) and the sensitivity of the detector (0.005 AU) we were able to detect 50 $ng.ml^{-1}$ which is largely sufficient for our system.

CONCLUSION

The continous sampling technique reported here allowed to obtain reproductible results for this new cephalosporin using very small sample volumes (7 µl). The most important interest was the inunterrupted determination and the direct and rapid dosage. Kinetics of the diffusion of the drug can be determined in vivo every fiveteen minutes.

In accordance with previous reports (5), no metabolites of cefsulodin were found in CSF or plasma samples in rats.

Other applications of this new techniques are possible : the "on line" determination of others drugs, endogene molecules such as cathecolamines. This technique can be improved maintaining temperature of the "micro vial" constant with a thermostatic circulating water bath. Furthermore the tubings and the vial can be protected against light when using photo-sensitive molecules.

In conclusion, the exemple of cefsulodin can be extended to other products without difficulties.

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