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HPLC determination of cefazolin in plasma, urine and dialysis fluid

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Abstract

Introduction

HPLC methods used in the analysis of cefazolin in biological fluids include solid phase extraction (Signs et al 1984), direct column injection (Moore et al 1991) and deproteination of plasma samples (Chan et al 1986). These methods involve complex sample preparation steps that lead to dilution, co-precipitation or instability of cefazolin thus, lowering the sensitivity of the assay. There is also little information about the determination of cefazolin in peritoneal fluid. The HPLC method proposed aims to improve the sensitivity of detection of cefazolin in plasma, urine and spent dialysate within acceptable accuracy and precision.

Method

(a) Plasma

0.5ml of plasma from volunteer donors was spiked with 1ml of cefazolin sodium standard solutions (1mcg/ml to 100mcg/ml). Complete deproteination of plasma was effected with 4ml of acetonitrile. Upon centrifugation at 3000rpm for 10min, an aliquot of the supernatant (0.6ml) was combined with 0.4ml of PABA (200mcg/ml) internal standard solution prior to analysis.

(b) Urine and Dialysate

5mg – 20mg of pure cefazolin sodium was dissolved in 5ml of urine/dialysate, to achieve a concentration range of 1000mcg/ml – 4000mcg/ml. 1ml of the spiked sample was diluted to obtain concentrations suitable for UV detection. 0.4ml of PABA (200mcg/ml) was then added to 0.6ml of the diluted sample prior to analysis. Blanks were prepared for each biological fluid and the samples were analyzed in triplicates.

A C18 Hypersil column (250x4.60mm) was used to effect separation. The mobile phase of 60% acetate buffer (pH4.0) and 40% acetonitrile was set at a rate of 1ml/min. and the injection volume was kept at 5μl. The absorbance of eluants was determined at 272nm.

Results / Discussion

Intra-day and inter-day analysis of plasma samples yielded %RSD values that fell below 1.0% (range: 0.0135 - 0.9416). The limit of detection has been determined to be 1mcg/ml.

Table 1: **Summary of % recovery in plasma, urine and dialysate samples.**

Biological Fluids	%R	
	Average	Range
Plasma	97.085	85.67 – 100.01
Urine	100.36	98.62 – 103.02
Dialysate	104.96	104.58 – 105.26

Figure 1 : HPLC separation of Cefazolin (100mcg/ml) and PABA (200mcg/ml) in plasma.

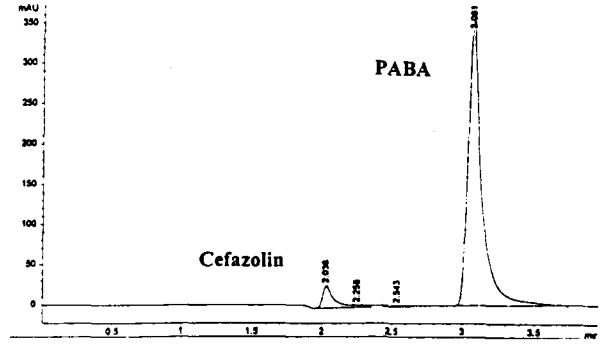
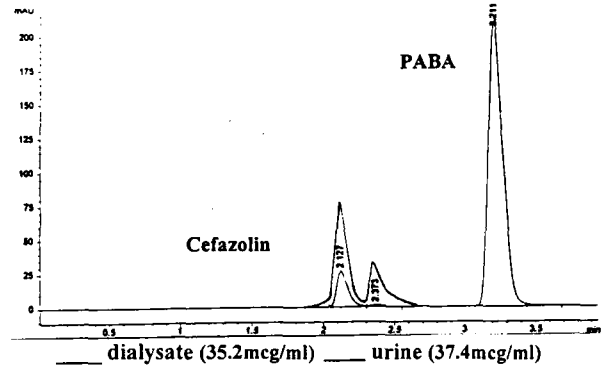


Figure 2 : HPLC determination of Cefazolin and PABA(200mcg/ml) in dialysate and urine.



____ dialysate (35.2mcg/ml) ____ urine (37.4mcg/ml)

Conclusion

The strength of the method lies in its ability to detect cefazolin in all the 3 types of biological fluids with accuracy, precision, reproducibility and sensitivity. The net analysis time for each sample is 4min, an advantage that allows the analysis of many samples within a short period of time. Furthermore, the sensitivity of the assay is excellent (1mcg/ml) eliminating the need for a concentration strategy.

References

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