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# Sensitive assay for the determination of cefazolin or ceftriaxone in plasma utilizing LC

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**Abstract**

A rapid, specific and very sensitive liquid chromatographic assay using standard ultraviolet detection has been developed to measure cefazolin (CFZ) or ceftriaxone (CFX) in small samples (200 μl) of plasma using either drug as the internal standard for measurement of the other. A rapid extraction was performed using $C_{18}$ bonded Sep Pak cartridges with high extraction efficiency for both drugs. The chromatographic system employed the use of a Nova-Pak $C_{18}$ 4-μm cartridge with a radial compression system preceded by a Guard-Pak with a $C_{18}$ insert. The mobile phase consisted of an aqueous solution containing 10 mM of dibasic potassium phosphate and 10 mM cetyltrimethylammonium bromide (pH 6.5) with acetonitrile (73:27 v/v). The drug and internal standard (CFZ/CFX) were detected using a UV detector set at a wavelength of 274 nm. Assay results were linearly related to the concentration ($r > 0.997$) for the wide range which was examined (0.005–120 μg/ml) for either drug. We report the precision, accuracy, recovery, linearity, sensitivity and specificity of this assay. The intra-run and inter-run CV was less than 9.02%. This method is currently being used for clinical therapeutic monitoring and pharmacokinetic studies of CFZ and CFX in patients undergoing cesarean section. 

*Keywords:* Cefazolin; Ceftriaxone; Drug monitoring; Liquid chromatography; Pharmacokinetics; Cephalosporins

## 1. Introduction

To prevent post operative infection in cesarean section patients, antibiotics have traditionally been given after cord clamping, with the disadvantage of not achieving adequate antibiotic levels in the mother until after the incision has been made [1–3]. Some recent investigations have claimed to find a higher degree of efficacy by administering drugs prior to the surgical opening, while exposing the infant to only low antibiotic levels [4,5]. Of two cephalosporins often given for this purpose, the third generation drug, ceftriaxone (CFX), is considered unique in that it has an extended half-life of 6–8 h [6,7] compared to 1.4 h

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for the first generation drug, cefazolin (CFZ) [8]. The two most common methods of analysis for cephalosporins in biological samples are microbiological assays [9,10] and liquid chromatographic (LC) procedures. The disadvantages of microbiological assays are the variability of precision and turnaround time as well as their lack of specificity resulting from possible interference from other antibiotics [11]. While a number of LC methods using UV detection have been developed for CFZ [12,13] and for CFX [11,14] in biological samples, to our knowledge none have been able to measure them both under the same extraction and chromatographic conditions, in part due to their wide difference in polarity. The assay described herein, was designed to be used for a study comparing the therapeutic efficacy of CFZ and CFX as a pre-operative prophylactic treatment against infection in Saudi Arabian women undergoing cesarean section procedures at this institution. As part of that study's design was to measure the exposure of the infants to these antibiotics, the assay needed to have a low detection limit to allow accurate determination of the drug levels in the infants for a period of 24 h after birth. The procedure described and validated in this study was designed to be a very sensitive and reproducible assay with a simplified extraction, using one as the internal standard (IS) for the other and employing standard, commonly available, LC equipment with UV detection.

## 2. Experimental

### 2.1. Chemicals and reagents

Cefazolin (sodium salt), ceftriaxone (sodium salt) and dibasic potassium phosphate (trihydrate) were supplied by Sigma (St Louis, MO, USA), and cetyltrimethylammonium bromide by Aldrich (Milwaukee WI, USA). Methanol, acetonitrile and 85% phosphoric acid were all of HPLC grade and supplied by Fisher Scientific (Fairlawn, NJ, USA). Our HPLC grade water was prepared by taking reverse osmosis water and passing it through a Milli-Q System (Millipore, Milford, MA, USA).

### 2.2. Instrumentation

The LC system employed consisted of a solvent delivery pump model 6000A, an Automated Gradient Controller Model 680, a Model 712 Ultra Wisp auto injecting module, a Guard-Pak precolumn module with Nova-Pak $C_{18}$ insert, an 8 mm $\times$ 10 cm Nova-Pak $C_{18}$ 4-μm particle size radial compression cartridge in conjunction with an RCM 100 radial compression module and a UV Detector Model 481 with the wavelength set at 274 nm. The detector was connected to a Data Module 746 integrator to store and print out the chromatographic data (all supplied by Waters Associates, Milford, MA. USA).

### 2.3. Mobile phase

The mobile phase consisted of a filtered aqueous solution containing 730 ml of 10 mM dibasic potassium phosphate and 10 mM cetyltrimethylammonium bromide with the pH adjusted to 6.5 with phosphoric acid and 270 ml acetonitrile, which was degassed immediately prior to use. The ion pairing reagent, cetyltrimethylammonium bromide, was used to prevent tailing of the highly polar CFX. The buffer was delivered at a programmed flow rate of 1.5 ml/min from 0 to 12 min, then increased using a linear gradient to 2.0 ml/min at 12.5 min, then further increased linearly to 3.0 ml/min at 13 min, and continuing at that rate until 26 min when the flow was decreased back to 1.5 ml/min, 1 min prior to the next injection.

### 2.4. Standard curves

Separate stock solutions with a concentration of 1 mg/ml for each drug were made in water, which could be stored in the dark at $-70$°C until needed. Under these storage conditions, the solutions could be kept for several months without measurable decomposition or reduction in peak height. No instabilities were observed for plasma samples kept under these same conditions. Fresh diluted stock solutions were prepared from the 1.0 mg/ml solutions weekly for each drug in water. To construct standard curves of 0.005, 0.01,

0.025, 0.05, 0.10, 0.25 and 0.50 μg/ml (low range) and 1.0, 5.0, 10.0, 20.0, 40.0, 80.0 and 120.0 μg/ml (high range) for both drugs, appropriate aliquots of diluted stock solutions of either CFX or CFZ were added to 200 μl of blank plasma. For the CFX standard curves, 50 μl of a 1.0 μg/ml (low range) or 50 μl of a 10 μg/ml (high range) stock solution of CFZ was added as internal standard. For the CFZ standard curves, 100 μl of a 1.0 μg/ml (low range) or 25 μl of a 100 μg/ml (high range) stock solution of CFX was added as the IS. These standard curves and the patient samples were extracted as described below.

### 2.5. Sample preparation

The plasma samples and standard curves were extracted using solid-phase $C_{18}$ bonded Sep-Pak cartridges (supplied by Waters Associates, Milford, MA, USA). Each cartridge was preconditioned by consecutively rinsing with first 5 ml of methanol, then 5 ml of water, after which the cartridge was dried for 1 min by light vacuum from the bottom. The unknown or spiked plasma samples with the appropriate IS were introduced to the top of the Sep-Pak cartridge which continued to have a light vacuum applied to it from the bottom. The cartridge then was washed with 3 ml water, dried under vacuum for 1 min and the drug and internal standard were eluted with 2 ml 60% methanol in water (v/v). This solution was completely evaporated under a gentle stream of nitrogen gas and the residue was reconstituted with 100 μl of LC mobile phase. A 75 μl aliquot of the reconstituted sample was injected directly into the LC system.

## 3. Results and discussion

To demonstrate the utility of the method in the lower range, Fig. 1 depicts three representative chromatograms including a blank plasma sample, a blank plasma sample supplemented with 0.25 μg/ml CFZ and a blank plasma sample supplemented with 0.25 μg/ml CFX. Fig. 2 depicts representative chromatograms in the higher range for a blank plasma, a blank plasma sample supplemented with 5 μg/ml CFZ, and a plasma sample collected from a patient 6 h after receiving a 1.0 gm dose of CFZ intravenously. As can be seen in these figures, the compounds were well resolved from each other with sharp peaks at 9.8 and 22.3 min for CFZ and CFX, respectively. It should be noted that overnight cleaning of the system with 0.3 ml/min 50% water and 50% methanol (v/v) significantly prolongs the life span of the pre-column Guard-Pak insert and analytical cartridge. The analytical column could normally be used without decreased performance for more than 300 samples, whereas changing of the pre-column insert was required after every 20–30 samples.

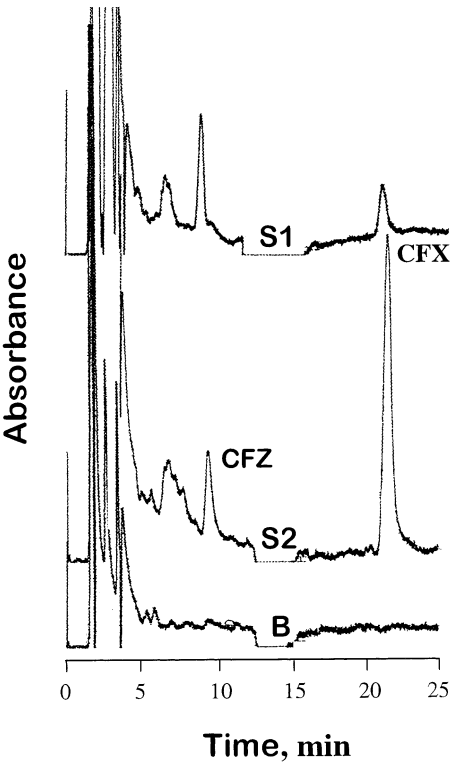


Fig. 1. Three representative chromatograms in the lower range including a blank plasma sample (B), a blank plasma sample supplemented with 0.25 μg/ml cefazolin (CFZ) and 0.5 μg/ml ceftriaxone (CFX) (S2) and a blank plasma sample supplemented with 0.25 μg/ml ceftriaxone with 0.25 μg/ml cefazolin (S1).

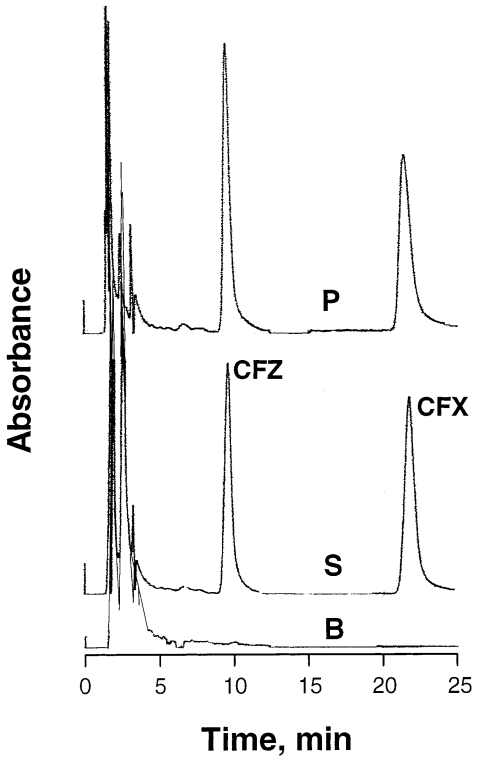


Fig. 2. Three representative chromatograms in the higher range including a blank plasma (B), a blank plasma sample supplemented with 5 μg/ml cefazolin (CFZ) and 12.5 μg/ml ceftriaxone (CFX) (S), and a plasma sample collected from a patient 6 h after receiving a 1.0 gm dose of CFZ intravenously, corresponding to 7.3 μg/ml CFZ with 12.5 μg/ml CFX as internal standard (P).

### 3.1. Linearity, detection limit and recovery

Standard curves for CFX were calculated by plotting the peak height ratio ($PHR_x$, peak height CFX/peak height CFZ) versus concentration for both the lower and higher ranges. Conversely, standard curves for CFZ were calculated by plotting the $PHR_z$ (peak height CFZ/peak height CFX) versus concentration for both the lower and higher ranges. The drug concentrations in unknown samples were calculated by use of these standard curves. There was an excellent linear relationship between the *PHR* of both lower and higher standard curves of CFZ and CFX using the formula:

$$Conc. = a + b(PHR)$$

where *Conc.* is the concentration of the drug measured, $a$ is the intercept, $b$ is the slope, and *PHR* is the peak height of the drug measured divided by the peak height of the IS. The mean (S.D.) correlation coefficient ($r$), $a$ and $b$ for the CFZ low range standard curve were 0.997 (0.0017), 0.032 (0.008) and 1.316 (0.347) while for the high range standard curve they were 0.999 (0.0007), 1.478, (0.688) and 3.678 (0.270), respectively. The mean (S.D.) ($r$), $a$ and $b$ for the CFX low range standard curve were 0.9974 (0.0018),

Table 1
Retention times of drugs which may be administered concomitantly with cefazolin or ceftriaxone in treatment of cesarean section patients

| Name of drug | Retention time, min |
|---|---|
| Acetaminophen | 2.3 |
| Amphotericin | 5.5 |
| Ampicillin | 18.2 |
| Atenolol | 1.5 |
| Bleomycin | 1.7 |
| Caffeine | 1.9 |
| Captopril | NDP[a] |
| Cefixime | 8.6 |
| Ceftazidime | 2.7 |
| Ceftriaxone | 23.1 |
| Cefazolin | 10.1 |
| Cephalexin | 7.8 |
| Ciprofloxacin | 2.5 |
| Digoxin | 1.7 |
| Diphenhydramine | 4.9 |
| Erythromycin | 4.6 |
| Ethambutol | 1.7 |
| Ethionamide | 5.0 |
| Heparin | 4.4 |
| Isoniazid | 1.7 |
| Kanamycin | 1.8 |
| Lidocaine | 11.3 |
| Metoclopramide | 1.9 |
| Metronidazole | 2.2 |
| Propofol | 1.2 |
| Propranolol | 4.0 |
| Rifampicin | 1.7 |
| Streptomycin | 1.8 |
| Theophylline | 2.4 |

[a] NDP, no detectable peaks were observed during the 26-min run under the conditions described.

Table 2
Analytical recovery, intra-run precision and inter-run precision of CFZ and CFX in plasma ($n = 7$)

| Drug | Amount added (μg/ml) | Amount[a] found (μg/ml) | Coefficient of variation (%) | Analytical[b] recovery (%) |
|---|---|---|---|---|
| Intra-run (same day) | | | | |
| CFZ | 100.0 | 101.50 (5.05) | 4.98 | 101.5 |
| CFZ | 0.2 | 0.188 (0.012) | 6.28 | 94.0 |
| CFX | 100.0 | 90.20 (4.43) | 4.90 | 90.2 |
| CFX | 0.2 | 0.178 (0.013) | 7.04 | 89.0 |
| Inter-run (different days) | | | | |
| CFZ | 80.0 | 80.43 (0.48) | 0.59 | 100.5 |
| CFZ | 0.10 | 0.107 (0.010) | 9.02 | 107.0 |
| CFX | 80.0 | 79.97 (2.38) | 2.97 | 100.0 |
| CFX | 0.10 | 0.094 (0.006) | 5.98 | 93.5 |

[a] Average (S.D.).
[b] Calculated as 100 × amount found/amount added.

0.037 (0.017) and 1.642 (0.409) while for high range standard curve they were 0.9977 (0.00128), −0.028 (0.257) and 0.143 (0.017), respectively. The limit of detection (LOD) under these conditions was found to be 0.005 and 0.003 μg/ml for CFZ and CFX, whereas limit of quantitation (LOQ) was found to be 0.015 and 0.010 μg/ml, respectively. Mean overall recovery of the drugs comparing peak heights from serum to aqueous solutions at the same concentration ranged from 93 to 95% for CFZ and from 95 to 97% for CFX at the concentrations of 0.5 and 5.0 μg/ml ($n = 6$ for each concentration tested).

### 3.2. Specificity and precision

The specificity of the described assay was investigated by determining the retention times of other drugs commonly used by patients undergoing cesarean section. Table 1 indicates retention times of these drugs run under the same conditions which are shown not to interfere with CFZ or CFX in this assay. To evaluate intra-run (same day) precision for plasma specimens, each of two different concentrations was analyzed, at 0.2 and 100 μg/ml. Table 2 shows the analytical recoveries, coefficient of variation (CV) for the intra-run (same day) precision and CV of the inter-run (different days) precision for the assay.

## 4. Conclusions

This report describes an accurate, highly sensitive and reproducible assay for CFZ or CFX in plasma using LC with a simple, expedient method of sample preparation. The small sample size of 200 μl used in this assay is an advantage for therapeutic monitoring of CFZ and CFX in maternal, cord and infant blood obtained at delivery, particularly as only a limited sample size may be available from the infant. The use of one drug as internal standard for the other makes the assay flexible as it is possible to perform accurate determinations of either drug without altering the chromatographic conditions or sample preparation. The described assay is currently being used to monitor the drug levels in both infants and mothers where either CFZ or CFX are being administered as pre-operative prophylaxis for cesarean section.

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