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Quantitation of cefazolin sodium in plasma and tissues by high-performance liquid chromatography

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Summary

A sensitive and reproducible HPLC method for the determination of cefazolin sodium in plasma and tissues of albino rats was developed. The assay technique utilizes a simple methanol extraction of the antibiotic and sulfamethoxazole or succinyl sulfathiazole as the internal standard. The proteins in plasma or tissue homogenates were precipitated by the addition of concentrated solution of trichloroacetic acid. Separation of the drug was performed on a μBondapak C_{18} column using a 0.1 M sodium acetate buffer (pH 3.85) : acetonitrile (89 : 11) mobile phase and the eluent was monitored at 254 nm. The limits of detection were 0.1 μg/ml of plasma and 1 μg/g tissue, and the linearity ranges were 0.1–200 μg/ml of plasma and 1–40 μg/g tissue. An excellent linear correlation was observed between the peak height ratios and the cefazolin concentration (r^2 = not less than 0.999). Minimum and maximum coefficient of variation were 2.54% and 3.12%, respectively, for the plasma and 2.17% to 3.51% and 0.82% to 3.06% for tissues at the concentration levels of 2 μg/ml (g) and 20 μg/ml (g), respectively. The method has been used to study cefazolin physiological pharmacokinetics in more than 80 rats and has been proven to be reproducible and sensitive.

Introduction

Several analytical techniques have been developed for quantitation of cefazolin sodium in aqueous solution. To date fluorometric analysis (Yu et al., 1977), quantitation based on reaction with hydroxylamine hydrochloride (Holl et al., 1975) and HPLC methods have been used to measure stability of cefazolin in aqueous solution (Yamana and Tsuji, 1976; Gupta and Stewart, 1980).

Establishment of a physiological pharmacokinetic model for cefazolin, in laboratory animals, requires a sensitive and specific assay procedure. The method must be quantitative and void of interference from plasma as well as useful in numerous tissues. Microbiological disc diffusion assays are tedious and lack the precision, specificity and sensitivity required for this type of model development. Additionally, the method is subject to greater error than that encountered in other pharmaceutical assays (Kuzel et al., 1969). The method developed by Wold and Turnspeed (1977), for determination of cefazolin in human serum, is not sensitive enough for pharmacokinetic studies.

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Recently an HPLC method has been described (Brisson and Fourtillan, 1981), which includes extraction and back extraction of cephalosporins from serum, prior to injection in the HPLC apparatus. In this method, sample treatment is time consuming, and is too tedious to be used for studies, such as physiological pharmacokinetic study, which requires lots of samples to be analyzed simultaneously. Furthermore, the method is not extended to biological tissues which requires workup of the tissue samples and requires detection of greatly reduced drug concentrations. We report on an extraction procedure coupled with a high-performance liquid chromatographic assay capable of the necessary speed, specificity and sensitivity required for optimum physiological pharmacokinetic model development.

Materials and Methods

Chemicals

Cefazolin as the sodium salt of 3-[[(5-methyl-1,3,4-thiadiazol-2-yl)-thio]methyl]-8-oxo-7-[2-(1H-tetrazol-1-yl)acetamido]-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid, was used as the commercial product (Kefzol, Eli Lilly). A stock solution of cefazolin sodium was freshly prepared by dissolving the powder in HPLC water at a concentration of 0.2 mg/ml. A stock solution of sulfamethoxazole (0.1 mg/ml) was prepared each day the assay was performed, and was used as the internal standard for plasma samples and all tissues except muscle. A stock solution of sulfasuxidine (1 mg/ml) was prepared by dissolving 10 mg in 0.25 ml of a 1% solution of sodium bicarbonate and diluting to 10 ml with HPLC water. A dilution to 0.05 mg/ml was made as necessary, and this solution was used as the internal standard for the assay of muscle tissue.

Mobile phase

A 0.1 M sodium acetate/acetic acid buffer solution at pH 3.85 was prepared using HPLC water and filtered through a 0.45 μm filter. The final mobile phase was prepared at 89% buffer solution : 11% HPLC acetonitrile and degassed by sonication and helium purge. The mobile phase was delivered into the HPLC apparatus at a rate of 2.0 ml/min.

Preparation of the plasma and tissue homogenates

Male albino Wistar rats were housed and fed for at least 3 days with standard laboratory chow before use (this procedure avoided extraneous peaks in the chromatograms). The rats were decapitated by guillotine and allowed to bleed into a beaker containing 0.2 ml of heparin solution (1000 IU). Blood was centrifuged at 3000 rpm for 15 min and plasma harvested. The following tissues were immediately collected in pre-weighted vials: heart, lung, liver, spleen, kidney, abdominal muscle and testicle. Each of these tissues was cleared of external blood by dipping in ice-cold water for 2–3 s and blotting dry on a filter paper. Tissues were homogenized in Tris buffer solution (pH 7.4) at a proportion of 4 ml to each g of tissue using a powered tissue homogenizer (Tissumizer, Tekmar). The tissues were homogenized while in an ice bath to avoid decomposition of cefazolin.

HPLC procedure

Separation was accomplished on an HPLC apparatus equipped with a model 6000 pump, a model U6K universal injector and a model 440 absorbance detector (Waters Associates, Milford, MA). The eluent was monitored at 254 nm and chromatographic peaks were recorded, and their respective heights integrated (Model 3390A, Hewlett Packard, Avondale, PA). The column used for separation was 10 μM octadecylsilane (30 cm $\times$ 4.6 mm i.d., μBondapak Waters Associates).

Determination of cefazolin in plasma

A standard curve was prepared from blank rat plasma, (0.2 ml in screw cap centrifuge tubes) containing aliquots of cefazolin sodium stock solution equivalent to 0.1, 0.2, 0.4, 0.8, 1.6, 2.4, 3.2, 4.0, 4.8 and 6.0 μg of cefazolin. Sulfamethoxazole internal standard solution (20 μl) was then added and the plasma sample diluted with 0.5 ml of HPLC water (this was done to prevent excessive retention times of endogenous plasma components that would otherwise interfere with the drug peak). While vortexing, at a low speed, 20 μl of 60% trichloroacetic acid (TCA)

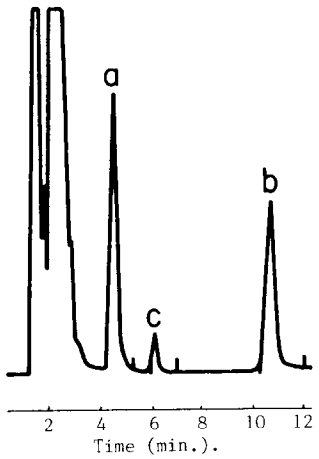

Fig. 1. Chromatogram of cefazolin in rat plasma. The cefazolin peak (a) had a retention time of 4.65 min. The sulfamethoxazole (internal standard) peak (b) had a retention time of 10.76 min. Chromatographic conditions were as described in the text. Peak (c) is of unknown plasma or tissue constituent and disappeared after treatment with methylene chloride.

solution was added to precipitate the plasma protein. Vortexing is necessary to distribute the acid rapidly and prevent formation of protein precipitate clots. 0.2 ml of HPLC methanol was then added to each tube and vortexed for 1 min at high speed. Tubes were centrifuged at 3000 rpm for 15 min and 100 μl of the supernatant was injected into the HPLC. Chromatogram of plasma sample from a rat injected with cefazolin is shown in Fig. 1. The plasma levels after intravenous administration of the drug into rats, via the tail veins (20 mg/kg body wt.) and decapitation after different time intervals, are presented in Fig. 3.

Fig. 2. Chromatogram of cefazolin in muscle tissue of Wistar rat. The tissue extract was treated with methylene chloride. The cefazolin peak (a) had a retention time of 4.65 min. The internal standard (sulfasuxidine) peak (b) had a retention time of 5.87 min.

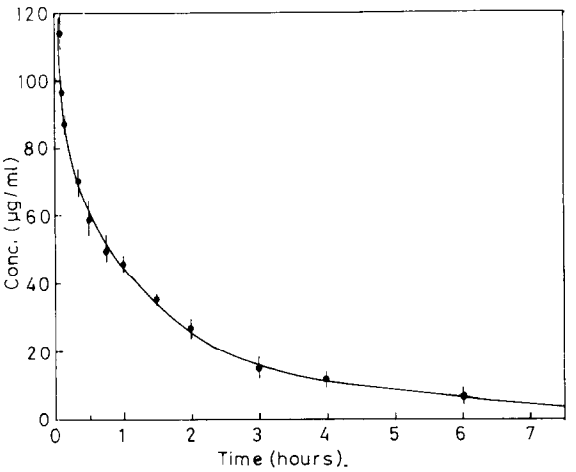

Fig. 3. Plasma levels of cefazolin sodium in Wistar rats, with renal dysfunction, after intravenous administration of the drug at a dose level of 20 mg/kg body weight.

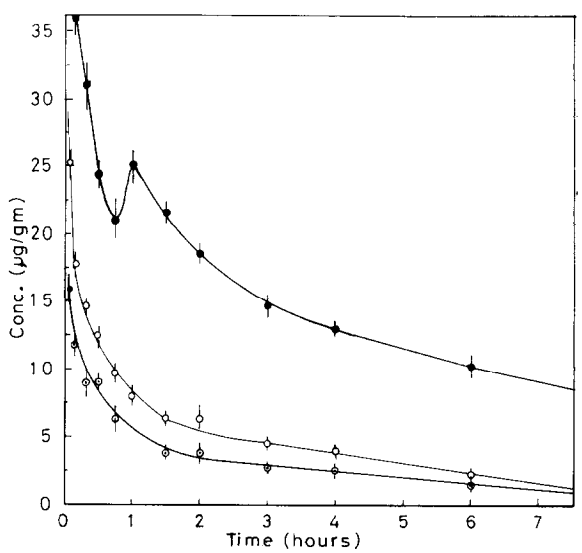

Fig. 4. Tissue levels of cefazolin sodium in Wistar rats, with renal dysfunction, after administration of the drug intravenously at a dose level of 20 mg/kg body weight. ○, heart; ⊙, muscle; ●, kidney.

Determination of cefazolin in tissues

One ml of the tissue homogenate was treated in the same way as plasma except that dilution with water was omitted, 25 μl of the 60% TCA solution was used and 0.25 ml of methanol was finally added. The supernatant liquid was transferred into another .tube and recentrifuged for 5 min. All tissues, except muscle tissue, were treated in this way. For muscle, 1 ml of homogenate was spiked with 20 μl of the sulfasuxidine internal standard solution. Precipitation of the protein and extraction of cefazolin were done in the same manner as the other tissues using 25 μl of 60% TCA solution and 0.2 ml methanol. The supernatant liquid was then decanted into another tube containing 4 ml of methylene chloride and vortexed for 1 min. Tubes were then centrifuged at 3000 rpm for 10 min and 100 μl of the supernatant aqueous phase was injected into the HPLC. For each tissue a standard curve was prepared by assaying blank tissue homogenate spiked with aliquots of cefazolin stock solution as used in plasma.

Chromatogram of cefazolin in muscle tissue of Wistar rat is shown in Fig. 2. The levels of cefazolin in different tissues of rats injected with the drug i.v., and decapitated at different time intervals, are presented in Fig. 4.

Results and Discussion

Tissues were homogenized in Tris buffer solution at a ratio of 1 g tissue to 4 ml buffer. This proportion was found reasonable for a homogenate liquid enough to be transferable and concentrated enough to detect cefazolin at as low a concentration as 1 μg/g tissue. The protein-precipitating agent, TCA, has been shown to decompose over time (Wheeler et al., 1980) and should be used within 3 h after preparation of its solution, otherwise, extraneous peaks may develop in the chromatogram. At the high concentration of TCA used, the decomposition problem was not seen as tested over 1 month storage. Only very small volumes of the concentrated TCA solution are used, for protein precipitation, which gives higher sensitivity to the method due to less dilution.

The optimum mobile phase was selected after testing various sodium acetate buffers (0.1 M) of different pH values. At pH 3.85, sharp peaks were obtained for both the drug and the internal standard. Retention times of the peaks vary with the concentration of acetonitrile in the mobile phase. Good separation of the drug, internal standard and extraneous plasma or tissue constituents was obtained by using a mobile phase containing acetonitrile 11% v/v.

When muscle tissue was treated in the same way as the plasma or the other tissues, an interference from endogenous component partially covered the peak of the drug. When the muscle tissue extract was vortexed with methylene chloride, the interfering endogenous component disappeared and complete separation of the drug could be obtained. Sulfamethoxazole is soluble in methylene chloride; therefore, it is unsuitable as the internal standard for muscle tissue-cefazolin determination. Sulfasuxidine was found to be more advantageous since it is insoluble in methylene chloride and has a retention time of 6.0 min which shortened the time of running each sample. However, it could not be used in samples not treated with methylene chloride because of an interference peak. Using the conditions given here, the retention time of cefazolin was 4.7 min, of sulfamethoxazole was 10.8 min and that of sulfasuxidine was 5.9 min (see Figs. 1 and 2). The method was linear for concentrations of cefazolin from 1 μg/ml (or g) to 40 μg/ml (or g) of serum (or tissue).

A concentration of 0.5 μg/ml of cefazolin in plasma could be easily determined by this method. However, by reducing the volume of water and methanol added to the sample (to half the volume) 0.1 μg/ml could be detected at signal-to-noise of approximately 4 : 1.

The ratio of methanol-to-water volumes used in the assay of plasma samples (0.2 ml for each 0.5 ml, respectively) was found to be optimum for obtaining a clear supernatant. Plasma samples containing cefazolin at concentrations above 30 μg/ml were diluted 5 times with HPLC water and assayed in the same manner described. A standard curve using blank plasma diluted 5-fold showed linearity over the concentration range 20 μg/ml to 200 μg/ml.

TABLE 1

PRECISION AND LINEARITY OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF CEFAZOLIN IN PLASMA AND TISSUES OF RATS

Tissue	(r^2) [a]	Slope	Precision at 2 μg/ml (g)			Precision at 20 μg/ml (g)		
			Mean [b]	S.D.	%RSD	Mean [b]	S.D.	%RSD
Plasma	0.9997	0.0786	1.9640	±0.0497	2.54	20.2617	±0.6320	3.12
Heart	0.9994	0.0718	1.9502	±0.0518	2.66	20.2719	±0.6210	3.06
Lung	0.9998	0.0750	1.9162	±0.0415	2.17	19.9659	±0.1793	0.90
Liver	0.9995	0.0814	2.0115	±0.1778	8.84	19.7492	±0.5948	2.96
Kidney	0.9999	0.0792	2.0162	±0.0708	3.51	19.9411	±0.1629	0.82
Spleen	0.9999	0.0783	2.0117	±0.0887	4.41	20.0460	±0.3699	1.84
Muscle	0.9996	0.0364	2.5034	±0.0587	2.32	19.8849	±0.4721	2.37

[a] r^2 = correlation coefficient.
[b] n = 6.

Table 1 shows the correlation coefficient and slopes of standard curves for cefazolin in plasma and various tissues of Wistar rats. At least 8 concentrations were used as spikes to create the standard curves (peak height ratios versus concentration). The relative standard deviation (RSD) percent was determined at concentration levels of 2 and 20 μg cefazolin per ml plasma or g tissue. The data shown in the table indicate a highly precise method for the determination of cefazolin sodium in plasma or tissues.

The techniques presented here for precipitating the protein and isolation of the drug from biological tissues are simple and rapid. A method with a high degree of accuracy and reproducibility has been developed. The method is being applied to the determination of cefazolin sodium in plasma and tissues of more than 80 rats in order to obtain a physiologically based pharmacokinetic model for the drug.

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