Simultaneous Determination of Cefazolin and Cefotaxime from Injections by Reverse Phase HPLC

V. M. SHINDE* AND C.V. SHABADI
Analytical Chemistry Laboratory, Department of Chemistry
The Institute of Science, 15, Madam Cama Road, Mumbai-400 032

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A new simple, precise, rapid and selective reverse phase high performance liquid chromatographic method is developed for the simultaneous determination of cefazolin and cefotaxime in injections. Microbondapak C-18 30 cm column is used as a stationary phase and 85:15:1.46 ml/L (Water:Acetonitrile:H_3PO_4) v/v and adjusted to pH 3.5 with triethylamine is used as a mobile phase. The flow rate is 1.5 ml/min and effluent is monitored at 254 nm. The method is linear in the concentration range of (40-200) mcg/ml for cefazolin and cefotaxime respectively.

CEFAZOLIN is a broad spectrum antibacterial drug closely related to penicillin[6]. Cefazolin is relatively well tolerated after either intramuscular or intravenous administration. Cefotaxime is the first of third generation cephalosporins and has been utilised effectively for meningitis caused by gram positive bacteria. Literature survey reveals many methods for determination of cefazolin by HPLC[1-5] and for determination of cefotaxime by HPLC[7-10]. However no attempt has been yet made for the simultaneous determination of cefazolin and cefotaxime from pharmaceutical dosage forms.

In this communication we propose a simple, precise and rapid HPLC method for the simultaneous determination of cefazolin and cefotaxime from injections. Standard samples of cefazolin and cefotaxime sodium were procurred from Lupin Labs. Limited, Mumbai.

Double distilled water and acetonitrile used were of chromatographic grade. Triethylamine, othophosphoric acid used were of A.R. grade. Samples of cefazolin sodium injections sterile Reflin (Ranbaxy) and cefotaxime sodium injections Omnatax (Hoechst India Ltd.) were procured from the market. A liquid chromatographic system consisted of a WATERS Isocratic 510 pump, with a 7125 Rheodyne valve injector, 20 microlitre fixed loop, equipped with a WATERS 486 UV detector controlled by Waters 745 Integrator was employed. Microbondapak C 18 (10 micron) 30 cm column was used as stationary phase.

*For correspondence

A standard solution of cefazolin and cefotaxime was prepared in mobile phase to have a concentration of 1 mg/ml respectively. This was used as a standard stock solution. A solution of para hydroxy phenyl acetamide was prepared by dissolving in 25 ml methanol and made up to the mark with mobile phase having concentration of 4 mg/ml. Calibration solutions were prepared by taking varying concentrations from 40 mcg/ml to 200 mcg/ml of standard stock solution in five different 50 ml volumetric flasks and 5 ml of internal standard stock solution was added in each flask and was diluted upto the mark with mobile phase. A 20 microlitre portion of the solution from each of the above flasks was injected and chromatograms were recorded.

The mobile phase used was 85:15:1.46 ml/L (Water:Acetonitrile:H_3PO_4) v/v and pH was adjusted to 3.5 with triethylamine. Flow rate was kept at 1.5 ml/min with an average operating pressure of 2200 psi and response was monitored at 254 nm. Five ml portions of these solutions were taken in 50 ml volumetric flasks, 5 ml of the internal standard stock solution was added and finally diluted upto the mark with the mobile phase. Twenty microlitres of the above solutions were injected and chromatograms were recorded. The amount of cefazolin and cefotaxime was calculated as reported in calibration procedure. To study the accuracy, reproducibility and

Table-1 : Recovery data of cefazolin and cefotaxime injections

Level No	Cefazolin Amount of Drug present in mg/inj	Cefazolin Amount of Standard Added in mg/inj	Total Amount* Recovered in mg/inj	% Recovery	Cefotaxime Amount of Drug Present in mg/inj	Cefotaxime Amount of Standard Added in mg/inj	Total Amount* Recovered in mg/inj	% Recovery
0	250	0	250.26	100.10	250	0	248.16	99.26
1	250	100	353.34	100.95	250	100	347.54	99.29
2	250	200	453.16	100.70	250	200	443.08	98.46
3	250	300	549.60	99.93	250	300	542.28	98.59
4	250	400	652.21	100.34	250	400	638.24	98.19
5	250	500	752.55	100.34	250	500	739.39	98.58

* Average of three experiments

precision of the above method and to check the interference from excipients used in the formulations in the above method, recovery experiment was carried out by standard addition method. Known amount of the standard cefazolin and cefotaxime solution was added to a fixed amount of sample solution at five different concentration levels. The recovery of the added standard was found out at five different levels. Each level was repeated three times. From the total amount of drug found, the percent recovery was calculated. Calibration curves were constructed for cefazolin and cefotaxime by plotting the ratio [peak area of drug] (Y-axis) against the amount of the drug concentration in mcg/ml (X-axis).

A quantity of injection equivalent to 250 mg cefazolin and cefotaxime was weighed and transferred into a separate 100 ml volumetric flask and 50 ml of mobile phase was added and the flask was kept for ultrasonication for 5 min, then it was diluted upto the mark with mobile phase. The above solution was filtered through a 0.45 micron whatmans filter paper using Milipore filtration kit. Five ml of the filtered solution of each was taken in a 50 ml volumetric flask, 5 ml of Internal standard stock solution was added and diluted upto the mark with mobile phase. Twenty-microlitres of this solution was injected and chromatograms were recorded. The amount of cefazolin and cefotaxime per injection was calculated from regression equation.

Various mobile phase systems were prepared and used for chromatographic separation, but proposed mobile phase comprising of 85:15:1.46 ml/L (Water:Acetonitrile:H_3PO_4) v/v and adjusted pH to 3.5 with triethylamine gave a better resolution of cefazolin, cefotaxime and para hydroxy phenyl acetamide (Internal Standard). The mobile phase composition was optimised. Under the described conditions the analyte peaks were well defined, resolved and free from tailing. The elution order was para hydroxy phenyl acetamide (3.04 min), cefotaxime (6.19 min) and cefazolin (7.67 min) at a flow rate of 1.5 ml/min.

A linear relationship was obtained for cefazolin and cefotaxime in the concentration range of (40-200) mcg/ml respectively. The calibration curves would be represented by following regression equations.

Y (cefazolin) = 0.385 x + 0.383 (r = 1.000)

Y (cefotaxime) = 0.184 x + 0.389 (r = 1.000)

These equations were used for direct evaluation of the drugs per injection. In replicate analysis (N=7) of the

injections the average content of cefazolin was 249.60 mg/inj (labelled amount 250 mg/inj) and relative standard deviation was ±0.147% respectively. The average content of cefotaxime was 249.68 mg/inj (lebelled amount 250 mg/inj) and relative standard deviation was ±0.191% respectively.

The results of the recovery analysis (standard addition) for cefazolin and cefotaxime are tabulated in table 1. From the results it can be revealed that thère is a good correlation between amount of standards added and total amount of drugs found at all concentration levels and hence it shows that there is no interference from the excipients used in the formulation. The mean recovery obtained for cefazolin and cefotaxime were between 99.93-100.95% and 98.46-99.29% respectively.

The proposed method is simple, precise and sensitive for simultaneous determination of cefazolin and cefotaxime from injections. The proposed method gives a good resolution between cefazolin and cefotaxime within a short analyses time (< 10 min) and is nowhere involves use of complex instruments or cubersome sample preperation. The proposed method is less expensive and takes much less time for the equilibration of the system as compared with the reported methods, therefore, it can be easily used for the simultaneous determination of these drugs from their injections.

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Pulse Polarographic determination of Dapsone in Tablets

D.K. SHARMA, N. VERMA, K. PRASHER AND JASVIR SINGH
Department of Chemistry, Himachal Pradesh University,
Summer Hill, Shimla 171005

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The sulphone group present in dapsone is smoothly and quantitatively reduced at dropping mercury electrode in dimethylformamide medium in the presence of pyridinium perchlorate, yielding a diffusion controlled wave at -1.50 V vs SCE, has been made the basis of present method. The determination has been made by normal pulse and differential pulse polarography by linear calibration plots.

DAPSONE, 4, 4-diaminodiphenyl sulphone is a drug used in the treatment of all forms of leprosy and dermatis herpetiformis. It is also a bacteriostatic against a wide range of bacteria, but mainly used against *Mycobacterium leprae*. The official (IP) method[1], involving nitrite titrations is not only semimicro but also nonspecific and is also used for the assay of other sulpha drugs. Other methods include HPLC[2,3], reversed phase HPLC[4] and bioassay using *Bacillus subtilis* BGA spores[5], but these methods have primarily been developed for the determination of residues of dapsone in foodstuffs such as milk, but no efforts have been made to analyse