ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
23 (2000) 307–313

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Determination of cefaclor by selective sample enrichment/clean-up on silica gel bonded polyelectrolyte in ion-exchange column chromatography

Sandeep Gupta, Bhim Bali Prasad *

Analytical Division, Department of Chemistry, Faculty of Science, Banaras Hindu University, Varanasi 221 005, India

Received 7 September 1999; received in revised form 4 January 2000; accepted 5 January 2000

Abstract

A silica gel-bound cationic polyelectrolyte, poly[*N*-chloranil *N*,*N*,*N*′,*N*′- tetramethylethylene diammonium dichloride], modified as ion-exchanger capable of molecular recognition of β-lactam antibiotic, was used in solid phase extraction through column chromatography for a sample clean-up and enrichment of analyte from a dilute solution. The optimum and selective sorption conditions for a model antibiotic, cefaclor, were established. The high selectivity of polymer at pH 9.5 and flow rate as high as 5 ml/min were observed for the quantitative sorption of cefaclor. The desorption by 0.1 N HCl at flow rate of 0.1 ml/min and subsequent heating at 80°C for 2 h allowed the antibiotic to be detected as corresponding oxazolone form in UV-spectrophotometric and differential pulse adsorptive stripping voltammetric measurements. The potential of the suggested approach was illustrated by estimating cefaclor in urine and blood plasma samples.

Keywords: Silica gel-bound polyelectrolyte; Ion-exchange column chromatography; Cefaclor; Differential pulse adsorptive stripping voltammetry; UV detection; Biological fluids

1. Introduction

The determination of β-lactam compounds in biological fluids by traditional methods has received much attention. For instance, cefaclor has been determined in biological fluids employing paper disk [1], thin layer chromatography [2], and microbiology [3,4] procedures. Direct estimations of optically active absorbing drugs such as ampicillin, cefoxitin and cephalexin have been performed by circular dichroism [5]. High performance liquid chromatographic (HPLC) analyses have been reported for evaluation of β-lactam antibiotics in human plasma, serum and urine by several authors [4,6,7]. The separation of various β-lactam antibiotics by reversed-phase HPLC was studied using C_{18} and phenyl-bonded columns [8]. The effects of ion-pairing reagents and mobile phase pHs on retention time in these columns have been investigated. An efficient microbore (0.3-mm internal diameter) HPLC column compatible with direct liquid interfacing

* Corresponding author. Fax: +91-542-317074.

0731-7085/00/$ - see front matter
PII: S0731-7085(00)00304-6

for mass spectrometric analysis of cephalosporins at ppb level has been designed [9].

Although the microbiological and paper disk methods are adequate for simple applications, they were found cumbersome for trace analysis. On the other hand column chromatographic analysis of target analyte in biological fluids suffers from two major problems: (1) column clogging and rapid deterioration (see Ref. [10] and references therein) in column performance by irreversible adsorption of the complex matrices such as protein, resulting in limited life time of the column, and (2) the undetectability of analyte by standard analytical technique due to its low concentration consequent upon the required dilution of the small volume of the biological fluid (blood plasma, blood serum, pus, saliva, urine etc.) available for sample handling. Both sample clean-up and enrichment would be feasible in one-column involving the solid-phase extraction procedure where the analyte is quantitatively sorbed on the stationary support duly immobilized with a suitable detection-sensitive reagent [10]. The approach is apparently much simpler than the two-column (i.e. pre-column derivatization and post-column separation) chromatographic procedure.

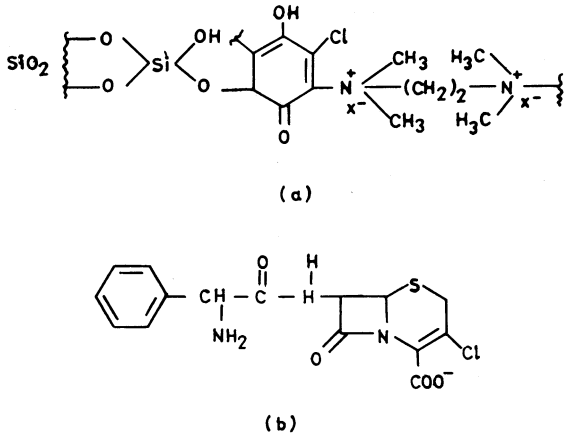

Fig. 1. (a) Structure of silica gel-bonded cationic polyelectrolyte (X^- = inter-exchangeable chloride and cefaclor anions). (b) Structure of cefaclor (anionic form).

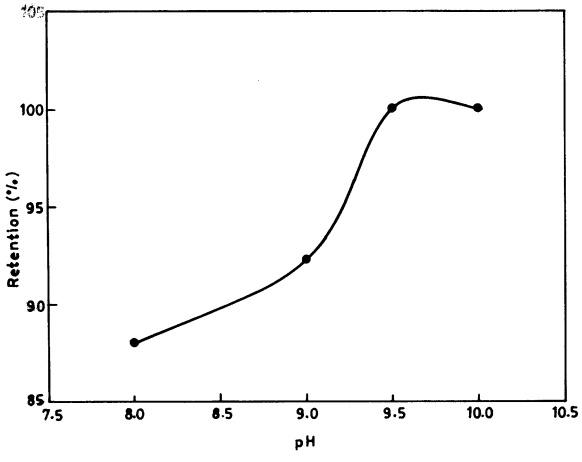

Fig. 2. Retention of cefaclor anion on silica gel-bonded cationic polyelectrolyte sorbent as a function of solution pH.

The present work describes the preparation of a typical silica gel-bonded polyelectrolyte tailor-made sorbent in chromatographic column which renders efficient sample clean-up and requisite preconcentration readily detectable from the UV and differential pulse absorptive stripping voltammetric (DPAdSV) techniques. Since the immobilization adopted in the present instance is simple recast from a solution of polymer, the sorbent preparation is convenient and fast. The column remains fresh for prolonged use as it always gets regenerated during desorption by hydrochloric acid and has high stability against any medium effect.

2. Experimental

2.1. Materials

All reagents were of analytical grade or better. Conductivity grade triple-distilled water was used for the preparation of stock solutions of cefaclor.

Silica gel (Merk TLC grade, mesh 60-20) was activated with concentrated HCl and HNO_3 acids and thoroughly washed with water, dried at $\sim$ 120°C for 12 h, cooled and stored in a desiccator.

The polymer, poly[*N*-chloranil *N*,*N*,*N*′,*N*′-tetramethylethylene diammonium dichloride] ($PCED(Cl)_2$), was prepared following a known method [11]. As is normal in a single batch, the equimolar toluene solutions of *N*,*N*,*N*′,*N*′-tetramethylethylene diamine (1.23 ml/10 ml) and chloranil (2.0 g/50 ml) were refluxed together for

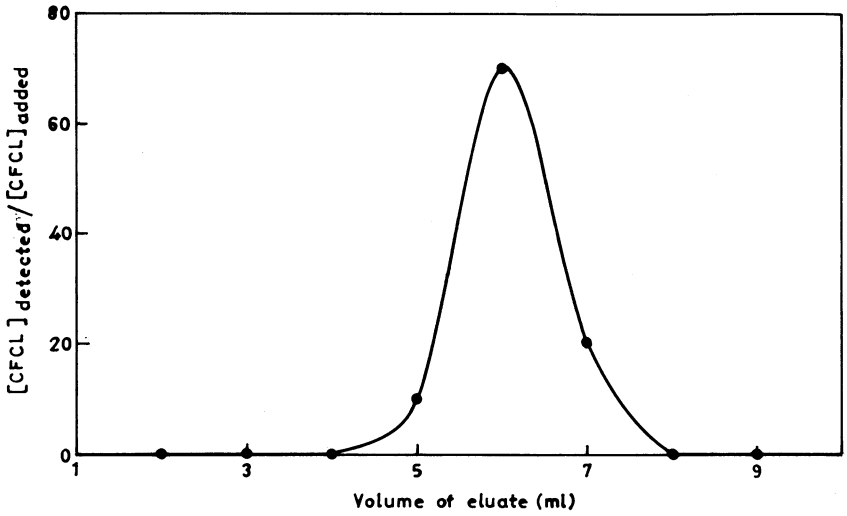

Fig. 3. Characteristic normal elution curve of cefaclor in 0.1 N HCl.

Table 1
Sample enrichment of cefaclor (CFCL) on silica gel-immobilized polyelectrolyte (silica-PCED($Cl)_2$) used as ion-exchange sorbent in chromatographic column

Sample	Detection technique	$[CFCL]_{in}$[a]	$[CFCL]_{out}$[b]	EF[c]	Recovery[d] (%)
Cefaclor as bulk (pure)	DPAdSV	4.5	157.3	35	96.6
	DPAdSV	56.0	1893.3	34	101.4
	DPAdSV	176.0	5666.7	32	104.9
	DPAdSV	278.0	9413.3	34	101.6
Urine (spiked)	DPAdSV	1.2	41.9	33	100.8
	DPAdSV	7.5	258.7	34	103.6
	DPAdSV	176.0	5652.2	32	104.6
	UV	0.7[e]	22.9[e]	34	101.5
	UV	1.0[e]	35.0[e]	35	103.6
Blood plasma (spiked)	DPAdSV	1.2	4.2	3	99.2
	DPAdSV	7.5	25.0	3	100.4
	DPAdSV	176.0	586.7	3	100.0
	UV	10.1[e]	33.8[e]	3	95.6
	UV	16.9[e]	56.3[e]	3	102.5

[a] Concentration of cefaclor (nM) in the 100-ml aliquot (pure and urine) and 10-ml aliquot (blood plasma) of a dilute solution in ammonia buffer (0.5 M, pH 9.5) passed at a flow rate of 5 ml/min.

[b] Concentration (nM) of desorbed cefaclor in the optimum volume containing the peak (3.0 ml). Desorption was done by passing a pH 1.0 mobile phase at 0.1 ml/min. The results are averages of five determinations with errors of less than ~5%.

[c] Enrichment factor, $[CFCL]_{out}/[CFCL]_{in}$.

[d] Recovery, (amount of desorbed CFCL)/(amount of CFCL in sample).

[e] Concentration in μM. Detection by UV method after required clean-up through column operation.

4 h and kept overnight. The resulting dark brown product, after washing with toluene and drying in vacuum, was characterised for elemental analysis (C = 29.4%, H = 6.1%, N = 5.6%, Cl^- = 3.58 mol/1000 g) corresponding to the structure, PCED($Cl)_2 \cdot 7(n+1)H_2O$; $n = 3$ (Fig. 1a).

To obtain the modified sorbent, a 4.0-g portion of silica gel was shaken for 2 h with 10 ml DMF solution (2.4×10^{-4} M) of PCED$(Cl)_2$ (3.5 mg). All DMF contents were evaporated off to get a brown-coloured coating of polymer on the surface of silica gel. The uncoated polymer was washed with DMF-water and dried in hot oven ($\sim$ 100°C). The extent of loading of polymer was determined to be 0.62 μM per g of silica gel.

2.2. Chromatography

The chromatography column was a Corning glass tube 13 cm long and 1.4 cm in diameter in which 2.0 g of prepared sorbent, slurried with water, was packed to a height of 3 cm supported by a G-1 sintered coarse glass frit fixed $\sim$2 cm above the drainage Teflon tap in the bottom of the column.

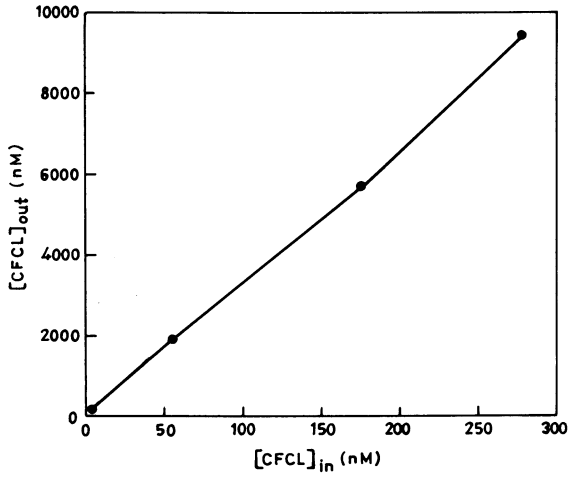

Fig. 4. Concentration of desorbed cefaclor ($[CFCL]_{out}$) versus concentration in the sample solution ($[CFCL]_{in}$) passed through the column containing silica gel-bonded cationic polyelectrolyte as sorbent. Conditions as described in Table 1.

Table 2
Direct analysis of cefaclor (CFCL) in urine and blood plasma[a]

Sample	Amount taken (μg/ml)	Amount found (μg/ml)	
		DPAdSV	UV
CFCL/urine	3.25	ND	4.15
	4.87	ND	6.36
CFCL/blood plasma	3.90	ND	4.66
	6.50	ND	7.38

[a] ND, not determined due to tensammetric DPdSV peak.

In the loading step, a 100-ml volume of test solution of appropriate pH (1.0 ml of test analyte plus 10 ml of 0.5 M ammonia buffer made from equal volumes of 1.0 M ammonia and 1.0 M ammonium chloride and diluted to 100 ml after adjustment of desired pH) was passed through the sorbent at a known rate (5 ml/min) via a peristaltic pump. The column effluent was directed to waste. Next, in the washing step, water was pumped through the column in order to rinse out the left-over solution between the particles of sorbent bed and in the tubings and fitting. Finally a pH 1.0 mobile phase (HCl solution, 10 ml) was used as eluent. This was pumped through the column inlet at a flow rate of 0.1 ml/min and effluents were collected in fractions of 1.0 ml in test tubes. The fractions were heated at 80°C on water-bath for 2 h and then subjected to monitoring through UV/DPAdSV techniques (see below).

The effect of pH on the retention of cefaclor was examined at a flow rate of 2 ml/min (the retention was only feasible in basic medium). The effect of flow rate on the sorption was studied over the range 1.5–5.0 ml/min. For an efficient sorption (100%) through the sorbent bed (2.0 g), the number of moles of cefaclor should be same or lower than the number of moles of counterions available for the ion-exchange [11].

2.2.1. Urine sample determination

In the urine sample determination, fresh human urine (89 ml) was diluted with ammonia buffer (0.5 M, pH 9.5, 10 ml) maintaining pH alkaline to that of buffer and finally spiked with known amount of cefaclor solution (1.0 ml). The sample loading and desorption were followed as discussed above.

2.2.2. Blood sample determination

Owing to the lack of available human blood plasma, the sample loading was restricted to 10-ml portion volume, unlike pure and urine samples. For this, 1 ml of blood plasma was spiked by

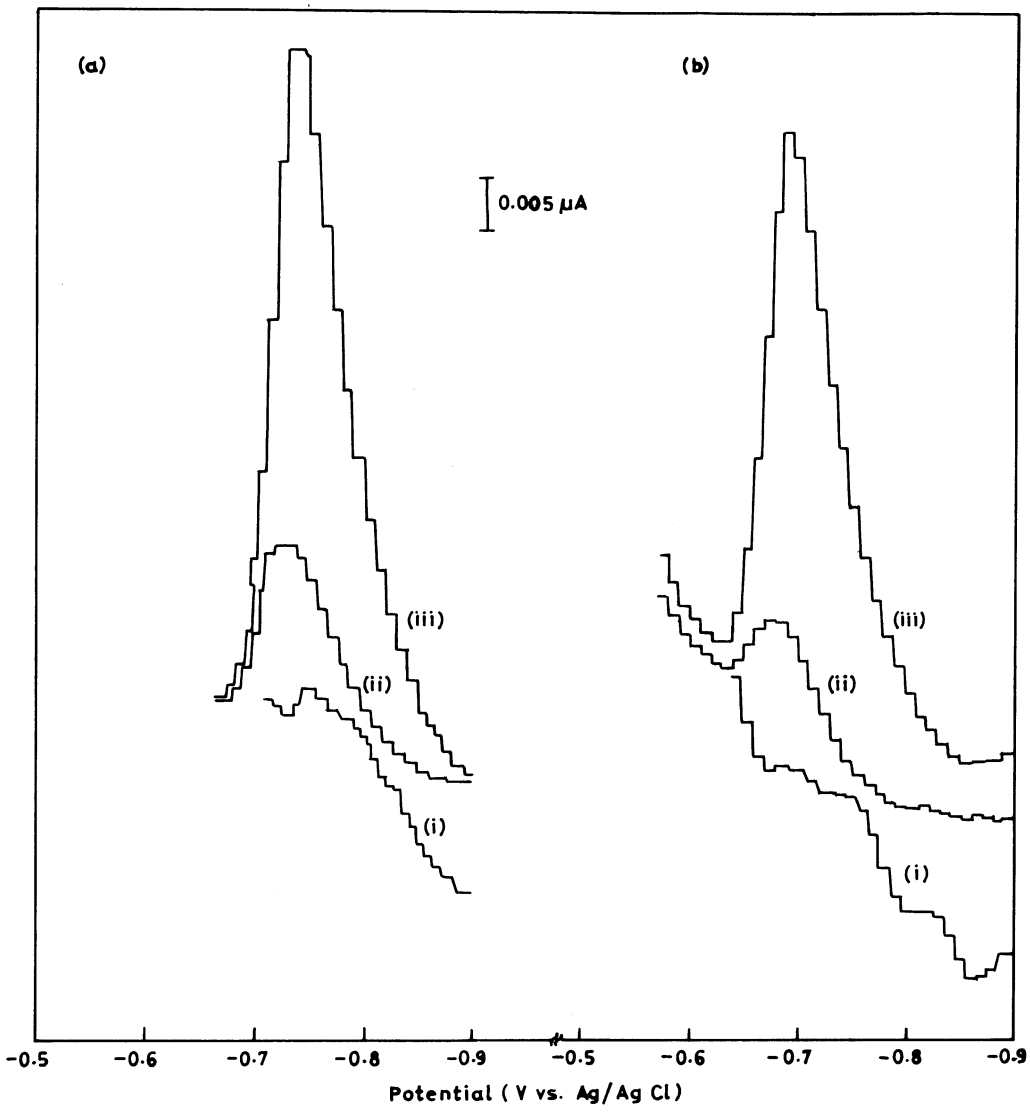

Fig. 5. DPAdSV curves for (a) human urine and (b) human blood plasma samples (KH_2PO_4-NAOH buffer, pH 8.0, $\mu = 0.1$ M; 120 s deposition time; scan rate 10 mV s^{-1}; pulse amplitude 25 mV). (i) Sample without passing through chromatographic column; (ii) sample after passing through chromatographic column; (iii) spike to (ii) by standard solution of cefaclor duly converted to corresponding oxazolone derivative.

the known amount of cefaclor (1.0 ml), diluted with 1.0 ml ammonia buffer (0.5 M, pH 9.5) and then the total volume was made up to 10 ml by water with setting of final pH close to 9.5. The sample loading and desorption were followed as discussed above.

2.3. Detection techniques

The UV absorption was measured at 340 nm against blank (0.1 N HCl) and the calibration plot with known amount of cefaclor over the range 0.40–7.29 μg/ml (1.04–19.96 μM) was made.

The conventional standard addition method was followed, as discussed earlier using a PAR model 264A voltammetric analyzer [12], for the determination of cefaclor under the optimized DPAdSV experimental conditions (40% ethanolic, KH_2PO_4-NaOH buffer, pH 8.0; ionic strength 0.1 M; accumulation time, 60 s; accumulation potential, -0.1 V vs. Ag/AgCl).

3. Results and discussion

The structure of silica-immobilized $PCED(Cl)_2$ (Fig. 1a) is confirmed through infrared spectrum of $PCED(Cl)_2$-immobilized pellet made from the powdered silica gel (diameter 10 mm and thickness 1 mm). The details of this are described in our earlier publication [11]. The immobilization proceeds with exchange of polymeric cation with the proton of weakly acidic site of $-SiOH$ group of silica gel involving $[SiO^-C^+]$ transitions (cf. a sharp absorption band [11] at 1092 cm^{-1}). The sorbent is highly stable at extreme pH range 2–10 without showing any leaching of polymer coating. The ion-exchange chromatographic retention of cefaclor (anionic form; Fig. 1b) takes place in a manner similar to the Stern-Gouy-Chapman electrical double-layer theory [13]. The retention effectiveness of the cefaclor anion is considered to be due to the cumulative effect of the ion-exchange interaction in the electrical double-layer model of stationary support and the π-electron overlapping between the aromatic rings of the polymer and analyte concerned. Fortunately, the sorbed analyte can readily be retrieved by eluent 0.1 N HCl without affecting the surface of ion-exchanger. In the chromatographic mode, the retention of cefaclor varied strongly with the mobile phase pH. While no retention was observed at acidic pH, cefaclor was found to be sorbed more in basic medium; cefaclor is almost quantitively sorbed (sorption 100%) in dynamic mode at pH ≥ 9.5 at flow rate ≤ 5 ml/min (Fig. 2). Interestingly, in the present instance, the column attained 100% sorption at flow rates ranging from 1.5 to 5.0 ml/min. However, a flow rate as high as 5.0 ml/min was selected for fast enrichment avoiding the probability of antibiotic degradation.

A dilute solution of cefaclor (1.0 μM) after loading at 5.0 ml/min was eluted with a pH 1.0 mobile-phase at a slow flow rate of 0.1 ml/min and then each eluted fraction (1 ml) was heated at ~ 80°C for 2 h to completely transform the analyte into the corresponding detectable oxazolone derivative [14] for DPAdSV monitoring. The normal elution curve is shown in Fig. 3. The initial four fractions (1 ml each) were dead volumes with no elution; however, the next three fractions caused a total 100% desorption of the sorbed analyte. The amount of eluted cefaclor, as obtained from the column, is divided by the optimum eluate volume (3.0 ml) within which the peak eluted. This amounts to the concentration of desorbed cefaclor ($[CFCL]_{out}$); the associated enrichment factor (EF) and the recovery are shown in Table 1. Apparently, the enrichment factor is constant over the range of sample concentration depending upon the ratio of the volume of test sample passed and the volume of eluate out from the column. The much smaller EF for blood plasma sample is primarily due to the very limited volume (1 ml) of the sample available for analysis. As is seen in Fig. 4, the concentration of cefaclor in the eluate, $[CFCL]_{out}$, increases linearly with sample concentration. This reveals the adsorption isotherm to be linear in the corresponding concentration interval.

In UV detection of cefaclor, the calibration curve assumed a linear expression, $A = 0.007 + 0.0365C$, where A and C correspond to absorbance and cefaclor concentration (μM), respectively. The correlation coefficient for five determinations was 0.993 and the molar absorptivity was found to be 4.39×10^4 M^{-1} cm^{-1}. The Sandell sensitivity (S)[1] was calculated as 0.013 mg cm^{-2} with a detection limit of 1.62 μg ml^{-1} (4.2×10^{-6} M).

The direct determination of cefaclor in body fluids by adsorptive stripping voltammetry may be hampered by interference effects from organic constituents in the sample matrices. Adsorption of

[1] $S = 10^{-3}/a$, where a is specific absortivity, i.e. absorbance of 1 μg/ml solution of determinant in cube with an optical path length of 1 cm.

surface-active compound onto working electrode usually causes peak-depression or even complete fouling of the electrode, and absorption/desorption process as such may yield distorted or tensammetric peaks. Furthermore, the direct UV detection of the sample yielded higher results (Table 2) and was observed to be as unreliable as direct DPAdSV technique. This is why the clean-up of body-fluids is required for accurate results (Table 1) with both techniques. Insofar as trace analysis at nanomolar level is concerned, the proposed solid-phase extraction in the present investigation, as shown in representative DPAdSV curves (Fig. 5), was found not only to be effective following clean-up, but could also enrich the sample after its selective isolation from complex matrices in one-column operation. Apparently, the subsequent chromatographic separation was not required. After passing the spiked samples of urine and blood plasma through the column, the results were found to be accurate and precise with recovery very close to that of pure sample (Table 1). This reveals the high level of selectivity attained in biological fluids with the present analytical scheme of column operation. One of the attractive features of this operation was that the metabolites (i.e. 7-amino-3-chloro-cepham-carboxylic acid (7-ACCA) and phenylglycine) [15] in anionic forms present in biological samples, despite the possibility of their ion-exchange co-sorption, could not be transformed into detectable species, i.e. respective oxazolone derivatives [14] on acidic hydrolysis and therefore this method could be said to be system-specific for cefaclor during DPAdSV as well as UV detection. Although the normal physiological concentrations in biological fluids are usually higher [6,7], the clean-up of biological fluids is necessary to mitigate the interferences posed from their respective complex matrices. Further, enrichment is also necessary after sample dilution for column operation and monitoring.

4. Conclusions

Antibiotic recognition in biological fluids by the use of polymer-modified silica gel ion-exchanger in column chromatography was found to be selective and sensitive with efficient sample clean-up without any problems of clogging or deterioration of the column.

Acknowledgements

Acknowledgement is made of the UGC sponsored Special Assistance Programme for instrumental facilities needed for this research. SG is thankful to Banaras Hindu University for a research fellowship.

References

[1] Y. Akimoto, Y. Mochizuki, A. Uda, H. Omata, J. Shibutani, H. Nishimura, M. Komiya, K. Kaneko, A. Fujii, Gen. Pharmacol. 23 (1992) 639–642.
[2] U. Ullmann, H.W. Dickmann, Infection 7 (1979) 554–556.
[3] Y. Akimoto, H. Nishimura, H. Omata, J. Shibutani, K. Kaneko, T. Kawana, T. Kaneda, H. Yamamoto, A. Fujii, Gen. Pharmacol. 27 (1996) 177–179.
[4] U. Ullmann, Zentralbl. Bakteriol. A 248 (1980) 414–421.
[5] P. Gortazar, M. Ravina, J.T. Vazquez, J. Pharm. Sci. 84 (1995) 1316–1321.
[6] K. Sato, K. Kobayashi, C.M. Moore, Y. Mizuno, Y. Katsumata, Forensic Sci. Int. 59 (1993) 71–77.
[7] P.M. Kovach, R.J. Lantz, G. Brier, J. Chromatogr. 567 (1991) 129–139.
[8] H.S. Huang, J.R. Wu, M.L. Chen, J. Chromatogr. 564 (1991) 195–203.
[9] C.M. Moore, K. Sato, Y. Katsumata, J. Chromatogr. 539 (1991) 215–220.
[10] R. Herraez-Hermandez, P. Campins-Falco, A. Sevillano-Cabeza, Anal. Chem. 68 (1996) 734–739.
[11] B.B. Prasad, A. Kumar, S. Sundd, React. Polym. 23 (1994) 229–238.
[12] S. Sundd, S.K. Prasad, A. Kumar, B.B. Prasad, Talanta 41 (1994) 1943–1949.
[13] T. Okada, Anal. Chem. 70 (1998) 1692–1700.
[14] R.J. Stoodley, Tetrahedron 31 (1975) 2331–2345.
[15] E. Harhausen, C. Simon, Infection 7 (1979) 603–605.