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Nanoscale packed capillary liquid chromatography–electrospray ionization mass spectrometry: analysis of penicillins and cephems

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ABSTRACT

A series of seventeen penicillins and cephems (cephalosporins and cephamycins) was examined by electrospray ionization. Separations by nanoscale packed-capillary liquid chromatography, with sub-microliter flow-rates, were performed using methanol–water and acetonitrile–water both containing trifluoroacetic acid gradients. In the on-column analyses, the protonated species usually predominate, and the fragment ions are often present which can be used for confirmation of compound identity. With combined nanoscale packed-capillary liquid chromatography–electrospray ionization mass spectrometry, separations and full-scan mass spectra can be obtained on *q12–15 ng of analyte, allowing the analysis of therapeutic levels of these antibiotics from only a few microliters of serum.

INTRODUCTION

Since the discovery of penicillin in 1928, penicillins and the structurally similar cephems, effective therapeutic agents against both gram-negative and gram-positive bacteria, have become important antibiotics for both human and animal use. Accurate and specific analytical techniques for these compounds are important in pharmacokinetic studies and clinical applications to determine safe and effective therapeutic levels of antibiotic [1–3]. The determination of drug levels in a particular body fluid might indicate potential clinical applications. For example, high levels in cerebrospinal fluid could indicate that a particular antibiotic might be useful in the treatment of meningitis [4]. Such methods should also be useful for studies of drug purity and stability [5,6], the examination of the mechanisms of drug resistance [7], and the determination of residues in food [8–11] as well as in clinical or forensic drug overdose studies.

Penicillins and cephems are highly polar and thermally labile, and most separations are now

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done by high performance liquid chromatography (HPLC) rather than by gas chromatography (GC) which usually requires a derivatization step [1]. There is a very extensive literature on the separation of these compounds by HPLC, including some recent review articles [1,12]. HPLC determinations, however, do not in themselves yield structural information on the particular analyte, and reliance on retention time alone for compound identification may lead to errors due to co-eluting compounds.

There are a few reports in the literature on combined liquid chromatography–mass spectrometry (LC–MS) of penicillins and related compounds, including papers on moving-belt LC–MS [13], particle-beam LC–MS [14], atmospheric pressure ionization (API) MS [15], continuous-flow fast atom bombardment (CF-FAB) [16], and thermospray (TSP) or plasmaspray (PSP) LC–MS [9–11,17–20]. Since the TSP nozzle is heated, however, TSP (or the related PSP) may lead to sample degradation or isomerization [17,20]. Electrospray, where the spray is generated by high voltage rather than by heat, is a gentle ionization technique [21,22], and in this paper, we have focused on the coupling of electrospray with nanoscale capillary liquid chromatography (nCLC) using 75 μm I.D. fused silica-packed capillaries.

In a conventional LC column, there are two distinct packing beds: a more densely packed region near the center of the column, and a less densely packed region near the wall, extending inwards about five particle diameters [23]. In our nCLC technique, where a 75 μm I.D. column is packed with 10-μm particles, the entire packing bed is uniform and is influenced by the wall effect [23,24]. Compared to conventional columns, these small columns have very high separation efficiencies and low mobile phase flow-rates. When coupled to the electrospray ionization (ESI) technique, all of the injected sample can be transferred to the mass spectrometer. Coupled with CF-FAB in our laboratory, these nCLC columns have been used for separations of peptides [25–29]. We have also coupled these columns with ESI and applied the combined technique to the separation and analysis of peptides [29] and antibiotics [30,31].

As part of our on-going interest in the applicability of nanoscale separation techniques combined with MS, we have investigated the separation and identification of seventeen penicillins and cephems by nCLC–ESI-MS. To evaluate the performance of these small packed capillary columns on separations of compounds extracted from a biological matrix, we analyzed therapeutic levels of a penicillin (piperacillin) and a cephem (cefotaxime) in serum. We report our results here.

EXPERIMENTAL

Chemicals

Structures, names, and abbreviations of the compounds studied are given in Table I. Standard samples of these compounds were obtained from the following companies, and were used without further purification. Cefmetazole (CMZ) was from Sankyo (Tokyo, Japan). Cefotiam (CTM), cefmenoxime (CMX), and sulbenicillin (SBPC) were from Takeda Pharmaceutical (Osaka, Japan). Ampicillin (ABPC), amoxicillin (AMPC), carbenicillin (CBPC), piperacillin (PIPC), penicillin G (PCG), cloxacillin (MCIPC), dicloxacillin (MDIPC), cefotaxime (CTX), cephalexin (CEX), cefoperazone (CPZ), cefoxitin (CFX), cephalothin (CET), and cefazolin (CEZ) were from Sigma (St. Louis, MO, USA).

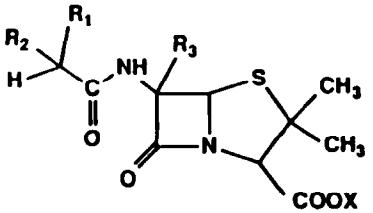
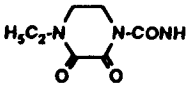
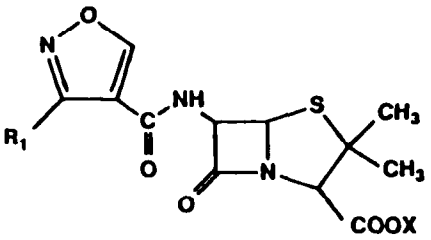
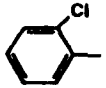
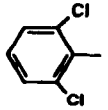
The mobile phase reagents used were HPLC-grade acetonitrile and methanol from Fisher Scientific (Fair Lawn, NJ, USA). Water used was from a Milli-Rho–Milli-Q system (Millipore, Bedford, MA, USA). The HPLC-grade acetic acid was from J. T. Baker (Phillipsburg, NJ, USA). Trifluoroacetic acid (TFA) was obtained from Fairfield (Blythewood, SC, USA).

Mass spectrometer and interface

The mass spectrometer–data system used was a VG 12-250 quadrupole mass spectrometer and data system (VG/Fisons, Altrincham, UK). The mass range scanned was from 100 to 1200 dalton at 2 s/scan for the nCLC work. Data were ac-

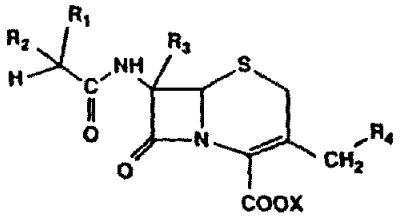
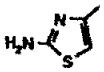
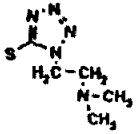
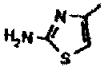
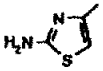
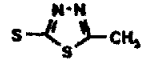
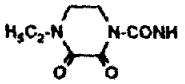
TABLE I

COMPOUNDS STUDIED

Abbreviation	Name	Molecular mass	Retention time[a] (min:s)	R_1	R_2	R_3	R_4	X[b]
Penicillins								
								
PCG	Penicillin G	334	19:02	Ph	H	H		K
ABPC	Ampicillin	349	18:51	Ph	NH_2	H		H
AMPC	Amoxicillin	365	15:08	PhOH	NH_2	H		H
CBPC	Carbenicillin	378	24:24	Ph	COOX	H		2Na
SBPC	Sulbenicillin	414	17:59	Ph	SO_3X	H		2Na
PIPC	*Pipericillin*	517	28:05		Ph	H		Na
								
MCIPC	Cloxacillin	435	33:35					Na
MDIPC	Dicloxacillin	469	36:11					Na

(Continued on p. 48)

TABLE I *(continued)*

Abbreviation	Name	Molecular mass	Retention time[a] (min:s)	R_1	$R_{2,2'}$	R_3	R_4	X[b]
Cephems								
								
CEX	Cephalexin	347	18:41	Ph	NH_2,H	H	H	H
CET	Cefalothin	396	27:08		H,H	H	$OCOCH_3$	Na
CFX	Cefoxitin	427	21:59		H,H	OCH_3	$OCONH_2$	Na
CTM	Cefotiam	525	14:13		H,H	H		Na
CTX	Cefotaxime	455	20:01		$=NOCH_3$	H	$OCOCH_3$	H
CMX	Cefmenoxime	511	20:11		$=NOCH_3$	H		Na
CEZ	Cefazolin	454	20:47		H,H	H		Na
CMZ	Cefmetazole	471	21:10	$N{=}CCH_2S{-}$	H,H	OCH_3		Na
CPZ	Cefoperazone	645	23:40		PhOH	H		Na

[a] Retention times from a separation done with an acetonitrile–water gradient (both containing 0.1% TFA). Gradient: 0–100% in 60 min. Flow-rate: ~550 nl/min.

[b] As purchased.

quired at a peak width setting of 1.15 in the VG Tune program, which gave a peak width at baseline of ~1.5 dalton.

The source used for the ESI experiments was a Vestec electrospray source, Model 611B (Vestec, Houston, TX, USA). Operating conditions were: needle voltage, ~3 kV; spray current, ~0.150 μA; block temperature, 266°C; chamber temperature, 55°C, skimmer, +12 V. The Vestec ESI interface does not rely on the use of a nitrogen curtain gas, but instead utilizes a heated block for desolvation [32]. The probe used was of coaxial flow design [30], which allows the introduction of additional solution (needed for spray stability) at the capillary tip without loss of chromatographic resolution.

nCLC system

The column preparation procedure has been described in detail elsewhere [28]. Briefly, after a frit has been made in the end of a 1.5–2 m × 75 μm I.D. × 150 μm O.D. fused-silica capillary, the first 20–30 cm is packed with 5- or 10-μm particles of a standard HPLC packing material. The empty portion of the column functions as a transfer line to the mass spectrometer. Packed with C_{18} particles, as in this study, the column functions as a standard reversed-phase column. For the separations of the β-lactams, 25 cm of the capillary was packed with AQ-C_{18} (YMC, Morris Plains, NJ, USA), and both acetonitrile–water and methanol–water (all containing 0.1% TFA) were used as mobile phases.

Injections onto the nCLC column were done by placing the end of the analytical capillary column into the sample vial, which contained an aqueous solution of the analytes and which was inside a stainless-steel pressure vessel. The sample was forced onto the column by pressurizing the vessel with helium for a given length of time [28].

In the nCLC system, the mobile phase flow through the analytical column was generated with a Gilson Gradient HPLC system and controller, operated at 0.8-1.0 ml/min. Flow through the capillary column was reduced by a factor of approximately 1000 with a splitting Tee. A 34 cm × 75 μm I.D. capillary was used to provide the backpressure needed to force approximately 0.5–0.8 μl/min of mobile phase through the capillary column.

At these flow-rates, a sheath flow was required for the generation of a stable spray. A Harvard syringe pump, Model 909 (Harvard Apparatus, South Natick, MA, USA) was used to generate the sheath flow, and was set to deliver ~8 μl/min. The sheath flow composition was methanol–3% aqueous acetic acid (50:50).

Serum extraction procedure

To evaluate the nCLC–ESI-MS techinque for the analysis of β-lactams in serum, a 1-ml aliquot of human serum was obtained. Control and spiked samples were prepared according to Annesley *et al.* [3]. The sample was spiked with 0.1 mg each of CTX and PIPC (to approximate a blood level of 100 mg/l) and extracted according to the procedure developed by Brisson and Fourtillan [33], as modified by Annesley *et al.* [3]. Following the extraction procedure in the same reference [3] (designed for HPLC–UV analysis), a 1-ml serum sample was spiked with the antibiotics (dissolved in 100 μl of water), then 1 ml of 0.4 *M* hydrochloric acid was added, and the solution was mixed. A 12-ml volume of chloroform–1-butanol (3:1, v/v) was added, and the vial was shaken for 10 min. The chloroform–butanol layer containing the antibiotics was first evaporated on a rotary evaporator to remove the chloroform, and the 1-butanol was removed by drying under helium. The sample was then reconstituted to the original 1-ml volume with water. (It should be noted that serum volumes of much less that 1 ml are necessary, since only a few microliters are actually needed for the analysis.)

RESULTS AND DISCUSSION

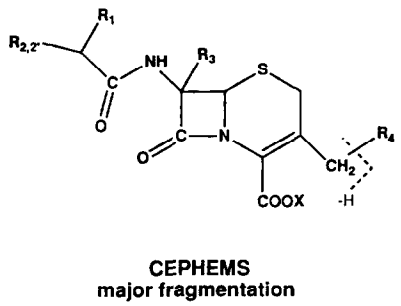
Mass spectra

In the on-column analyses, protonated molecular ions usually predominate. Even at low skimmer voltages, however, significant fragmentation occurs and can be useful for structure determination or confirmation. A particularly useful fragmentation of penicillins is the cleavage across the

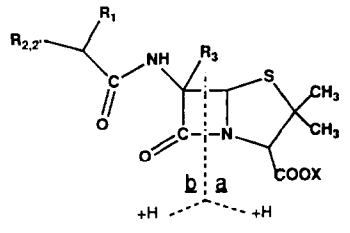
β-lactam ring, to give $[b+H]^+$, and the loss of the R_4 group from the protonated molecule (for those cephems where R_4 is not a hydrogen atom) (Fig. 1). These appear to be fragmentation reactions and not thermal degradations. Ions such as the loss of 26 from the protonated molecules of ABPC and penicillin V, observed by Siegel *et al.* [20] and attributed by him to thermal degradation in the TSP vaporizer, were not observed in the ESI spectra of β-lactams, either by us or by other researchers [34]. Fragmentations of these molecules are quite complex, and some may be collision-induced. These fragmentation reactions are the subject of an ESI-MS–MS study now in progress [35].

nCLC separations of standards

Separations were performed in methanol–water and acetonitrile–water (both mobile phases containing 0.1% TFA). Cephems and penicillins, being ionic and amphoteric compounds, are often separated with ion-pair reagents (which are often incompatible with LC–MS), or by reversed-phase HPLC with an ionic buffer to suppress ionization and increase retention times [1,11]. In a study of ion-pair reagents and mobile phase pH on the HPLC retention of five β-lactams, the authors found that the greatest retention in the absence of ion-pair reagents occurred at pH 2.5, near the pK_a values, where the –COOH groups were half ionized [36]. This was the lowest pH studied by this group. For our separations, we used a pH of ~1.5 to further suppress ionization of these compounds.



CEPHEMS
major fragmentation



PENICILLINS
major fragmentations

Fig. 1. Structures of penicillins and cephems, showing characteristic fragmentations.

Since the detection limits of penicillins and cephems are not particularly low by ESI (these compounds are at least an order of magnitude less sensitive than sulfonamides [31] or macrolides [30]), a preconcentration technique was used where the samples were dissolved in water, and fairly long injection times, *e.g.* 2–4 min (corresponding to 1.6–3.2 μl), were used to concentrate the samples on the front end of the column. After the sample was loaded onto the column, a gradient was used to elute the compounds.

Peak shapes and sensitivities were significantly better with the acetonitrile–water (TFA) mobile phase than with the methanol–water mobile phase, so only the acetonitrile–water (TFA) results are shown here. Representative separations and spectra obtained from the acetonitrile–water (TFA) are shown in Figs. 2 and 3 for a mixture of eight penicillins. Separations and the on-line spectra obtained from a mixture of nine cephems are shown in Figs. 4 and 5. All of the compounds could be separated by simple linear gradients, and both 30- and 60-min gradients could be used. Figs. 2–4 were obtained with 30-min gradients; a mixture of all seventeen compounds was separated using a 60-min gradient, and the retention times of all of the components are given in Table I.

The levels of β-lactams in the solutions used to obtain the chromatograms and spectra in Figs. 2–5 were on the order of 10–125 μg/ml, so that the concentrations of these drugs in the solutions injected approximate reported therapeutic blood

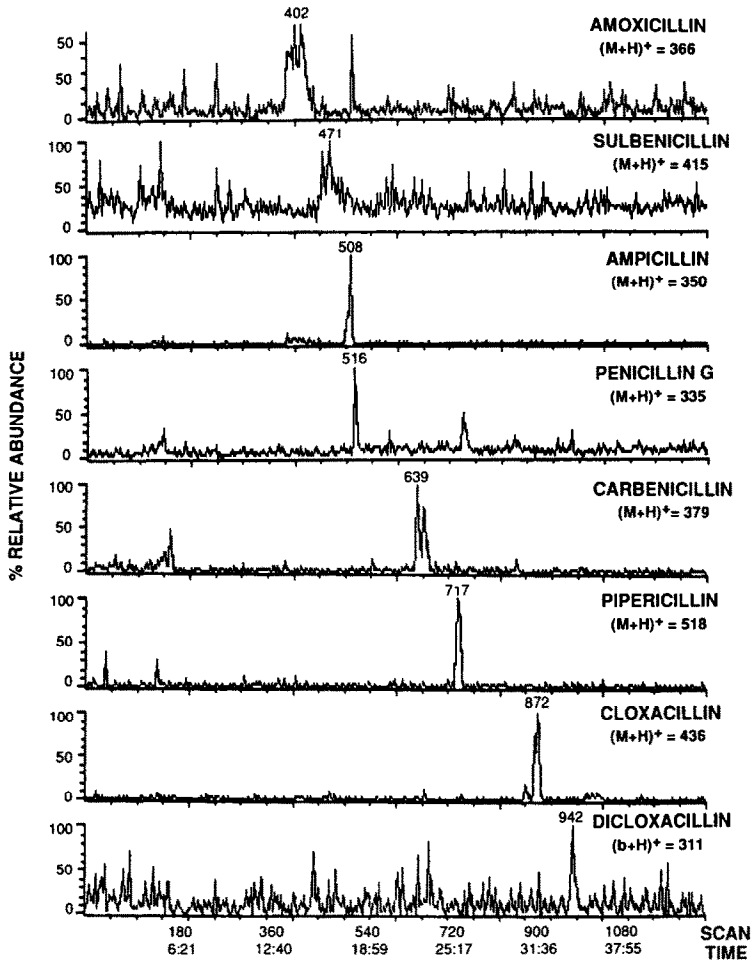


Fig. 2. Reconstructed ion chromatograms for characteristic ions obtained from a mixture of eight penicillins, using an acetonitrile–water (both containing 0.1% TFA) gradient. Gradient: 0–100% in 30 min. Flow-rate: ~725 nl/min. Amount injected: ~500 pmol of each component.

levels of 50–200 mg/l [3]. Only ~2 μl of these solutions (the equivalent of ~25–250 ng per component) were used to produce the chromatograms and spectra shown in these figures. It should also be noted that full mass range scanned data (*m/z* 100–1200) were obtained at these concentrations. Full-scan detection limits are about ten to twenty times lower than the chromatograms shown, and vary from compound to compound, partly due to differing extents of fragmentation and variations in chemical noise (from mobile phase background ions) at certain masses. Estimated full-scan detection limits are ~2–15 ng. If needed, detection limits for these compounds could be lowered by ~two orders of magnitude by selected-ion monitoring (SIM) [14].

Of the seventeen β-lactams studied, only SBPC did not exhibit good peak shape, possibly because the sample may be partially ionized when dissolved in water. AMPC also gave a fairly broad peak. Loading the samples onto the column as a mixture in 0.1% TFA instead of water did, in fact, improve the peak shape for AMPC, but the peak shape of SBPC was still not improved, perhaps because it is the only compound

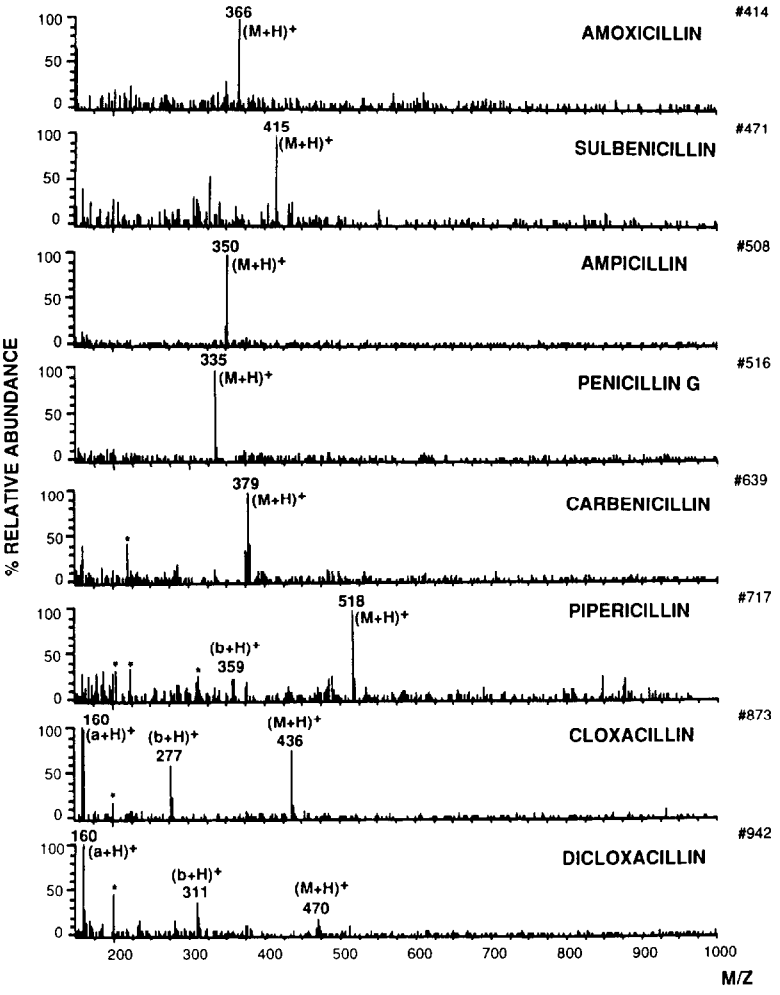


Fig. 3. On-line ESI mass spectra obtained for the eight penicillins from the separation shown in Fig. 2. Ions at m/z 199, 224, 288, and 376 are incompletely subtracted background ions, marked with an asterisk in the spectra.

with an $-SO_3H$ group, which is a stronger acid than –COOH. In nCLC, the injection solvent is more critical than in conventional HPLC, where the injection solvent is only a small portion of the mobile phase flow. In nCLC, the injection solvent is, in effect, the initial mobile phase. Thus, if a compound is not retained at the front end of the column, it will start to move during the injection, resulting in a broad peak.

The utility of the fragment ions is demonstrated in the analysis of the penicillin mixture. In Fig. 2 the $[b + H]^+$ ion from MDIPC has been used to identify this compound. Both MCIPC and MDIPC exhibited protonated molecules, but there was an impurity in the mixture (a chlorinated compound with an apparent protonated molecule at m/z 468) which gave a second peak in the reconstructed ion chromatogram for dicloxacillin (Fig. 6). Thus, the $[b + H]^+$ ion from MDIPC was more specific for this compound than was the protonated molecule. It should also be noted that the ^{37}Cl isotope of this impurity might have given a "false positive" for MDIPC even by LC–MS, had the analysis been done with insufficient chromatographic resolution and SIM on only the protonated molecule.

One of the penicillins, PCG, showed two peaks in the reconstructed ion chromatographic (RIC)

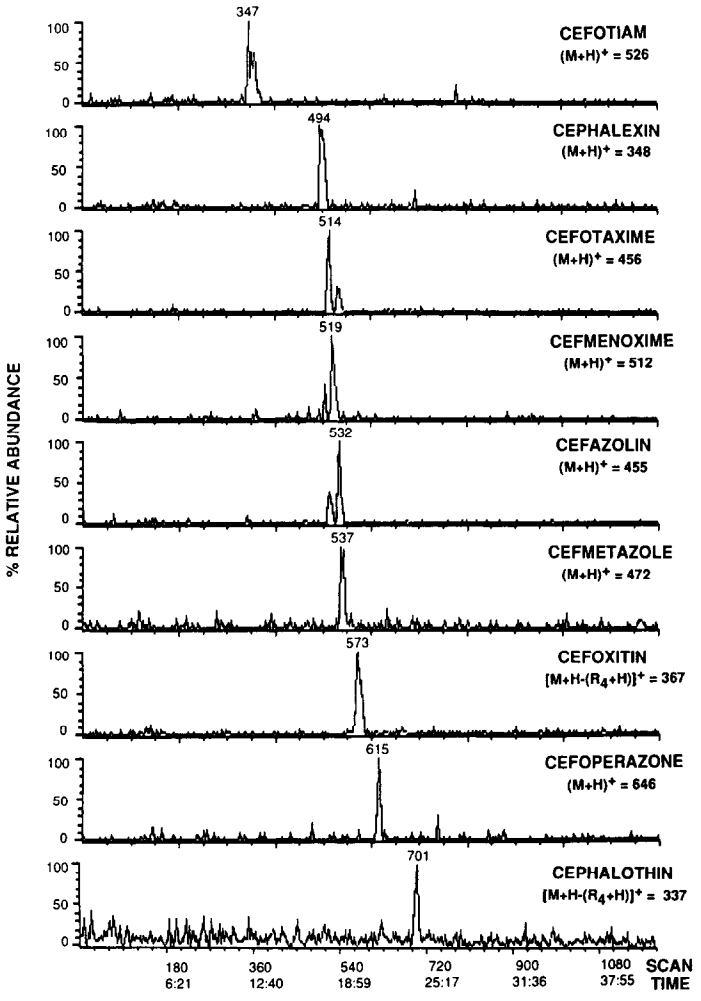


Fig. 4. Reconstructed ion chromatograms for characteristic ions obtained from a mixture of nine cephems using an acetonitrile–water (both containing 0.1% TFA) gradient. Gradient: 0–100% in 30 min. Flow-rate: ~725 nl/min. Amount injected: ~500 pmol each of CTM, CMZ, CMX, CFX, CPZ and CEZ, and ~60 pmol each of CEX, CTX, and CET.

trace (Figs. 2 and 7). The earlier-eluting peak dominates for injections of fresh solutions, while the later-eluting peak is larger for older PCG solutions. These two compounds, probably isomers, both show $[M + H]^+$ ions at m/z 335, while the degradation product exhibits more cleavage of the lactam ring to give both the $[a + H]^+$ ion at m/z 160 and the $[b + H]^+$ ion at m/z 176. This degradation product may be one of those reported in HPLC–UV degradation studies of PCG [4,5]. The $[a + H]^+$ ion is at the same mass, m/z 160, for all of these penicillins, and many of the other components can be seen in the RIC trace for this ion, indicating that this ion may prove useful in screening or as a confirmatory ion for many of these compounds. Of the nine penicillins and degradation products in this mixture, only AMPC and undegraded PCG did not undergo this fragmentation and gave no peaks in the RIC trace for m/z 160.

In the on-column studies of cephems, CFX and CET did not exhibit abundant protonated

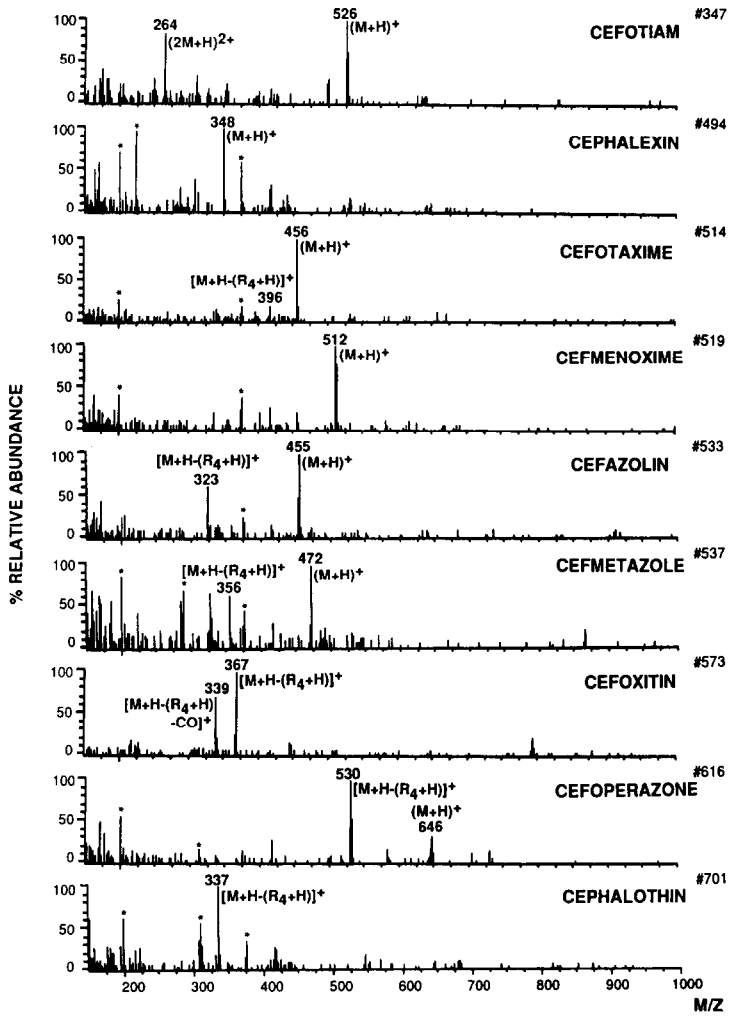


Fig. 5. On-line ESI mass spectra obtained for the nine cephems from the separation shown in Fig. 4. Ions at m/z 199, 224, 288, and 376 are incompletely subtracted background ions, marked with an asterisk in the spectra.

molecules, so the $[M + H - (R_4 + H)]^+$ ions at m/z 367 and 337, respectively, were used to obtain the chromatograms shown in Fig. 4. The utility of the $[M + H - (R_4 + H)]^+$ fragment ion for analyte confirmation is demonstrated by comparing the results for CTX and CEZ. The molecular masses of these compounds differ by 1 dalton, and they only differ by a few scans in retention time. For these compounds, the $[M + H - (R_4 + H)]^+$ ions at m/z 396 and 323, respectively, can be used for confirmation.

Serum analysis

A 60-min linear gradient was used for the serum analyses. The reference chromatograms shown in Fig. 8a are the RIC traces for the protonated molecules of CTX and PIPC from a mixture containing all seventeen standards. The serum was spiked at the 100 mg/l level with PIPC and CTX (approximating therapeutic levels). A 3-min injection of the extract produced the chromatogram shown in Fig. 8b. This 3-min injection corresponded to an injection volume of 2.4 μl

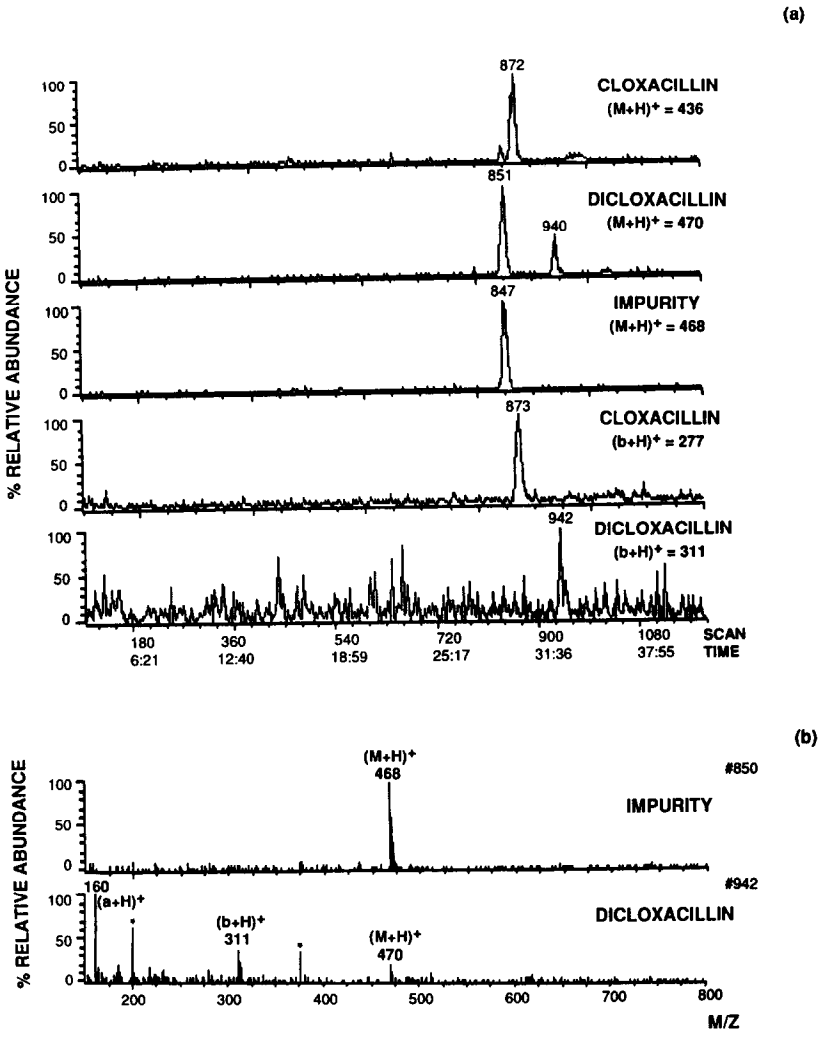


Fig. 6. (a) Reconstructed ion chromatograms for the $[M + H]^+$ and $[b + H]^+$ ions from MCIPC, MDIPC, and the m/z 468 impurity peak. (b) ESI mass spectra for the impurity peak and MDIPC. The ions at m/z 199 and 376 in the MDIPC spectrum are incompletely subtracted background ions, marked with an asterisk in the spectrum. Separation was done with an acetonitrile–water (both containing 0.1% TFA) gradient. Gradient: 0–100% in 30 min. Flow-rate: ~725 nl/min.

(equivalent to 2.4 μl of the original serum). While amounts of human serum many not be limiting, the extraction procedure used should also be applicable to rat serum, where small injection volumes may be necessary.

Both peaks in the serum sample are a few scans later than the corresponding peaks in the reference standard mixture, probably due to the long injection time. If only UV detection had been used, the CTX peak might have been mistaken for CMX or CEZ (Table I). If nCLC–ESI-MS analysis is used, however, the combination of mass spectrum and retention time unequivocally identifies the compound.

The serum analysis was included in this study because in neither of our previously published papers on nCLC–ESI-MS [30,31] did we attempt to determine the antibiotics in a biological fluid. Since there are only a few milligrams of actual packing material in these columns, there was the possibility that matrix components might plug the column, or coat the packing and degrade the

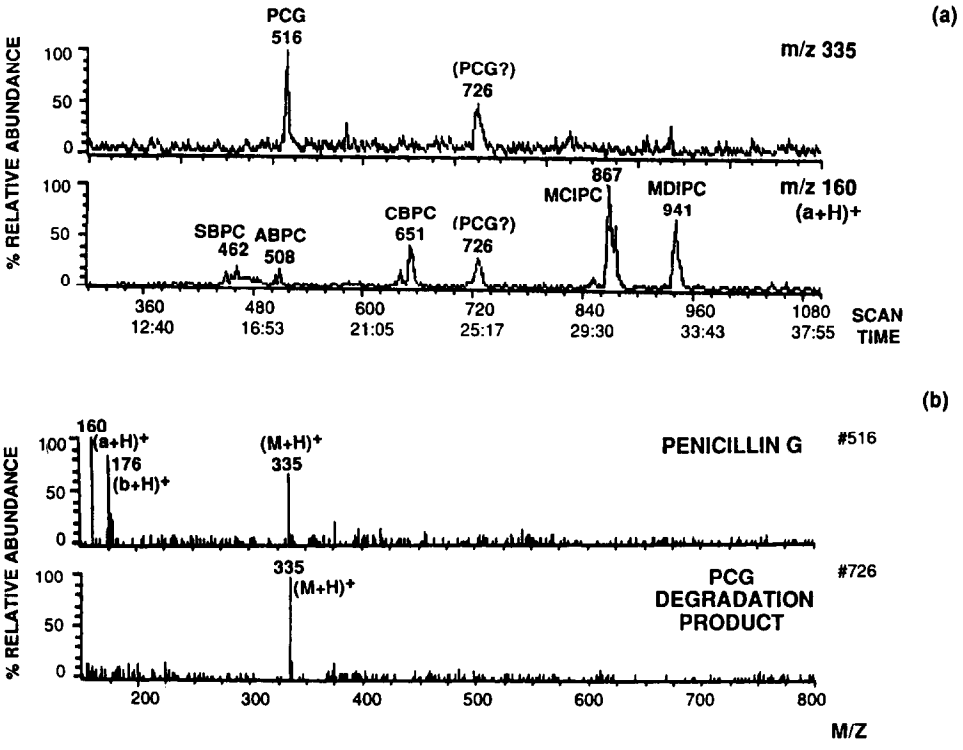


Fig. 7. Reconstructed ion chromatograms and spectra of PCG and its degradation product. (Conditions as in Fig. 2.)

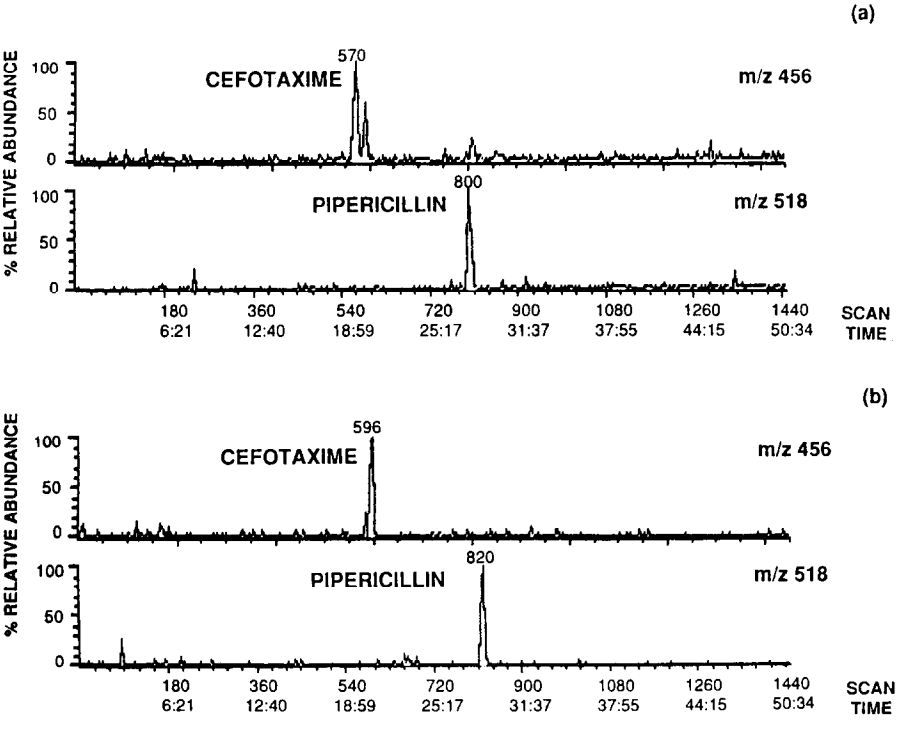


Fig. 8. Reconstructed ion chromatograms for the $[M + H]^+$ ions of CTX and PIPC from (a) a mixture of all seventeen penicillins and cephems and (b) an extract of human serum, spiked at the 100 mg/l level. Separations were done with an acetonitrile–water gradient. Gradient: 0–100% in 60 min. Flow-rate: ~550 nl/min.

separation — problems we had encountered with an earlier microcolumn technique, open tubular liquid chromatography. These difficulties did not arise with the nCLC technique.

CONCLUSION

In summary, the combined technique described in this paper utilizes the most sensitive ionization technique yet reported for these compounds, and couples it with a separation technique where all of the injected analyte is delivered to the MS source, without need for a split. We have also demonstrated a separation technique applicable to many members of this class of compounds, and compatible with LC–MS analysis. Nanoscale LC–ESI-MS has proven to be very useful for the separation of penicillins and cephems. All of the compounds studied, with the exception of SBPC, gave satisfactory on-line separations and ESI mass spectra, and these separations and full-scan mass spectra can be obtained from as little as 60 pmol of material. Separations are reproducible, and the protonated molecules and characteristic fragment ions assist in analyte identification.

REFERENCES

1 M. C. Rouan, *J. Chromatogr.*, 340 (1985) 361.
2 A. Lau, M. Lee and R. Sharifi, *Int. J. Clin. Pharmacol.*, 23 (1985) 391.
3 T. Annesley, K. Wilkerson, K. Matz and D. Glacherio, *Clin. Chem.*, 30 (1984) 908.
4 L. Balant, P. Dayer and R. Aukenthaler, *Clin. Pharmacokin.*, 10 (1985) 101.
5 J. M. Blaha, A. M. Knevel and S. L. Hem, *J. Pharm. Sci.*, 64 (1975) 1384.
6 J. M. Blaha, A. M., Knevel, D. P. Kessler, J. W. Mincy and S. L. Hem, *J. Pharm. Sci.*, 65 (1976) 1165.
7 K. O'Hara, Y. Shiomi and M. Kono, *FEMS Lett.*, 24 (1984) 291.
8 H. Terada, M. Asanoma and Y. Sakabe, *J. Chromatogr.*, 318 (1985) 229.
9 R. D. Voyksner, K. L. Tyczkowska and A. L. Aronson, *J. Chromatogr.*, 567 (1991) 389.
10 K. L. Tyczkowska, R. L. Voyksner and A. L. Aronson, *J. Chromatogr.*, 490 (1991) 101.
11 K. L. Tyczkowska, A. L. Aronson and R. D. Voyksner, *J. Vet. Pharmacol. Ther.*, 14 (1991) 51.
12 M. Margolis, *Adv. Chromatogr.*, 28 (1989) 333.
13 M. A. McDowall, D. E. Games and J. L. Gower, *Int. J. Mass Spectrom. Ion Phys.*, 48 (1983) 157.
14 C. R. Blakley, J. C. Carmody and M. L. Vestal, *Clin. Chem.*, 26 (1980) 1467.
15 M. Sakairi and H. Kambara, *Anal. Chem.*, 60 (1988) 774.
16 R. M. Caprioli and S.-N. Lin, *Proc. Natl. Acad. Sci. U.S.A.*, 87 (1990) 240.
17 Y. Ohki, T. Nakamura, H. Nagaki and T. Kinoshita, *Nippon Iyo Masu Supekutoru Gakkai Koenshu*, 15 (1990) 201.
18 S. Suwanrumpha and R. B. Freas, *Biomed. Environ. Mass Spectrom.*, 18 (1989) 983.
19 S. Suwanrumpha, M. A. McLean, S. W. Fink, C. Wilder, K. Stachiwiak, D. F. Dyckes and R. B. Freas, *NATO ASI Ser., Ser. C*, 353 (1992) 281.
20 M. M. Siegel, R. K. Isensee and D. J. Beck, *Anal. Chem.*, 59 (1987) 989.
21 M. Dole, L. L. Mack, R. L. Hines, R. C. Mobley, L. D. Ferguson and M. B. Alice, *J. Chem. Phys.*, 71 (1979) 4451.
22 C. M. Whitehouse, R. N. Dreyer, M. Yamashita and J. B. Fenn, *Anal. Chem.*, 57 (1985) 675.
23 R. T. Kennedy and J. W. Jorgenson, *Anal. Chem.*, 61 (1989) 1128.
24 K. E. Karlsson and M. Novotny, *Anal. Chem.*, 60 (1988) 1662.
25 L. J. Deterding, M. A. Moseley, K. B. Tomer and J. W. Jorgenson, *Anal. Chem.*, 61 (1989) 2504.
26 M. A. Moseley, L. J. Deterding, K. B. Tomer and J. W. Jorgenson, *J. Chromatogr.*, 480 (1989) 197.
27 L. J. Deterding, M. A. Moseley, K. B. Tomer and J. W. Jorgenson, *J. Chromatogr.*, 554 (1991) 73.
28 M. A. Moseley, L. J. Deterding, K. B. Tomer and J. W. Jorgenson, *Anal. Chem.*, 63 (1991) 1467.
29 L. J. Deterding, C. E. Parker, J. R. Perkins, M. A. Moseley, J. W. Jorgenson and K. B. Tomer, *J. Chromatogr.*, 554 (1991) 329.
30 C. E. Parker, J. R. Perkins, K. B. Tomer, Y. Shida, K. O'Hara and M. Kono, *J. Am. Soc. Mass Spectrom.*, 3 (1992) 563.
31 J. R. Perkins, C. E. Parker and K. B. Tomer, *J. Am. Soc. Mass Spectrom.*, 3 (1992) 139.
32 M. H. Allen and M. L. Vestal, *J. Am. Soc. Mass Spectrom.*, 3 (1992) 18.
33 A. M. Brisson and J. B. Fortillan, *J. Chromatogr.*, 223 (1981) 393.
34 R. D. Voyksner and T. Pack, *Rapid Commun. Mass Spectrom.*, 5 (1991) 263.
35 C. E. Parker, Y. Shida, K. O'Hara and K. B. Tomer, in preparation.
36 H.-S. Huang, J.-R. Wu and M.-L. Chen, *J. Chromatogr.*, 564 (1991) 195.