Journal of Pharmaceutical & Biomedical Analysis
Vol. 3, No. 1, pp. 87–94, 1985
Printed in Great Britain

0731–7085/85 $3.00 + 0.00
Pergamon Press Ltd.

Short Communication

Fourier transform mass spectrometry of cisplatin and analogues

ROBERT R. WELLER,[1] JOHN R. EYLER[1] and CHRISTOPHER M. RILEY*[2]

[1] *Department of Chemistry, Leigh Hall, University of Florida, Gainesville, FL 32611, USA and*
[2] *Department of Pharmaceutics, J. Hillis Miller Health Center, University of Florida, Gainesville, FL 32610, USA*

Keywords: *Cisplatin; platinum complexes; Fourier transform; mass spectrometry.*

Introduction

In 1967 Rosenberg *et al.* [1] described the ability of *cis*-dichlorodiammineplatinum(II) [$PtCl_2(NH_3)_2$], (CDDP, cisplatin) to inhibit the division of *E. coli* cells which continued to grow into long filaments. These observations [1] indicated the potential usefulness of cisplatin and other platinum complexes in the treatment of cancer. Following successful animal studies and clinical trials cisplatin has become one of the most widely used drugs for the treatment of those solid tumours which are resistant to other forms of therapy [2–6]. Over two thousand analogues of cisplatin have been synthesized and tested [2, 7] in attempts to increase the cytotoxicity and reduce the severe side effects, which include kidney damage, hearing loss and gastrointestinal disturbances [2, 7–14].

Structure–activity relationships [2, 7, 10–15] have shown that platinum(II) complexes are in general more active than similar platinum(IV) complexes [12–15]. A notable exception to this is *cis*-dichloro-*trans*-dihydroxybis-2-propanamineplatinum(IV) (CHIP), which has significant antineoplastic activity [14, 15]. Platinum complexes can undergo nucleophilic substitution reactions [16] in aqueous solutions and it is believed that these reactions account for the mode of interaction with DNA, which in turn leads to the inhibition of cell division. The reactions of cisplatin with DNA and its sub-units (nucleotides etc.) have been studied extensively [2, 7, 17–22] and are well characterized. However, the analogous reactions with endogenous nucleophiles, which are believed to be responsible for the biotransformation of platinum complexes, are less well understood [2, 18, 23–25].

Previous studies [2, 18, 23–25] indicate that the biotransformation of cisplatin is extremely complex *in vivo*, since it is found to react *in vitro* with a wide variety of

* To whom correspondence should be addressed.

endogenous substances such as methionine [18], methionine-containing peptides and proteins [23], and other divalent sulphur-containing compounds [25]. The complexity of the *in vivo* transformation of platinum complexes is highlighted by the work of Riley *et al.* [18, 23], who have separated up to ten platinum-containing species produced by the chemical reactions which follow the incubation of cisplatin with single sulphur-containing nucleophiles.

Following an intravenous infusion of cisplatin there is an initial, short-lived and rapid decline in the total plasma concentration of platinum, followed by a slower biphasic elimination [26, 27]. The platinum species of low molecular weight (<25,000) are eliminated rapidly from plasma (half life, 30 min) and fall below the limit of detection by flameless atomic absorption (50–100 ng/ml) within 2 hr [26]. Over this time period, about 60–80% of the non-protein bound platinum can be accounted for by the intact drug [26]. However, the identities of the complex or complexes which comprise the remaining fraction of unbound platinum are unknown.

The low molecular weight platinum complexes are eliminated in the urine [26, 28] and the ratio of intact drug to total filterable platinum reflects that found in the plasma [28]. LeRoy *et al.* [24] have used cation exchange chromatography to separate two platinum-containing fractions of urine collected from patients receiving cisplatin. However, the structures of the complex or complexes found in these fractions were not elucidated.

Structural identification of platinum biotransformation products has been hindered by the lack of specific analytical methodologies having the requisite sensitivity [17]. In particular, platinum complexes are generally involatile and unstable at moderate temperature (<250°C) and thus unsuitable for analysis by mass spectrometry. Although a secondary-ion mass spectrum of the 2:1 adduct of methionine:platinum has been reported [18], this technique has not been generally applied in this type of study.

The present studies are concerned with investigating the potential and limitations of Fourier Transform Mass Spectrometry (FT-MS) [29–31] as an alternative to conventional mass spectrometry for the analysis of platinum complexes. FT-MS is a relatively new technique based on the older ion cyclotron mass spectrometer [29–31]. Unlike conventional mass spectrometers which separate events in space, all events in the FT-MS spectrometer occur in a small cyclotron analyzer cell and are hence separated in time. The increased sensitivity of FT-MS compared with conventional techniques arises from the much lower operating pressures employed (10^{-8} compared with 10^{-4} torr), which permit the easier volatilization of solutes with low vapour pressures. In a conventional mass spectrometer, only a small fraction of the ions produced are actually detected. In contrast, virtually all the ions produced in FT-MS are trapped in the analyzer cell and detected. Approximately 10^4–10^5 ions can be detected in the analyzer cell and further sensitivity is gained in the FT experiment by exploiting the multiplexing advantage and by signal averaging techniques.

Experimental

Powdered samples of the compounds examined (Fig. 1) were kindly provided by Dr. A.J. Repta (University of Kansas, Lawrence, KS), who had received them as a gift from the National Cancer Institute, Bethesda, MD, USA. Spectra were collected on a Nicolet model FT/MS 1000 Fourier Transform ion cyclotron resonance mass spectrometer (Nicolet Analytical Instruments, Madison, WI, USA). Samples located in 2-mm capillaries at the end of a solid insertion probe were inserted through the standard

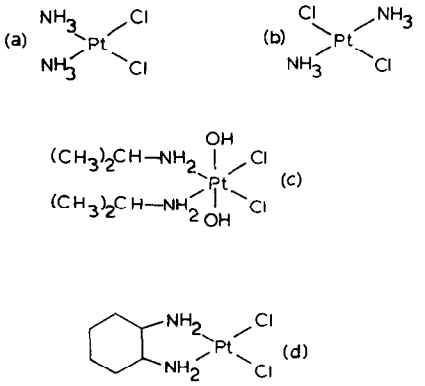

Figure 1
Structures of the platinum complexes studied: (a) *cis*-dichlorodiammineplatinum(II) (Cisplatin); (b) *trans*-dichlorodiammineplatinum(II); (c) *cis*-dichloro-*trans*-dihydroxybis-2-propanamineplatinum(IV) (CHIP); (d) *cis*-dichlorocyclohexyl-1,2-diammineplatinum(II).

airlock. Background pressure before sample introduction was *ca* 10^{-9} torr. After sample introduction a base pressure of about 2×10^{-8} torr was obtained before data collection. The sample was heated gradually until a rise in pressure to about 10^{-7} torr was observed (at *ca* 275°C).

Ionization was accomplished by electron impact (EI) with a 50 eV electron beam for positive ions, with no delay before the excitation–detection sequence. Negative ions were formed by electron capture (EC), employing 0.2 eV electrons produced with a 25 ms beam, with a delay of 100 ms introduced before the excitation–detection sequence to allow time for the electron capture. Standard (broadband) spectra were obtained using 16,000–32,000 data points with one order of zero filling. High resolution spectra (narrowband) were obtained by heterodyning a single frequency reference signal with the ion signal, filtering out the sum frequencies and digitizing the difference frequencies. In all cases 1000 transients (spectra) were collected before transformation. The signal-to-noise ratio was usually better than 100:1 for the broadband spectra and *ca* 20:1 for the high resolution spectra.

Results and Discussion

A positive ion spectrum obtained with cisplatin is shown in Fig. 2. Clusters of peaks were observed for the parent ion $[PtCl_2(NH_3)_2]^+$ (*m/z* 298). The sequential loss of the

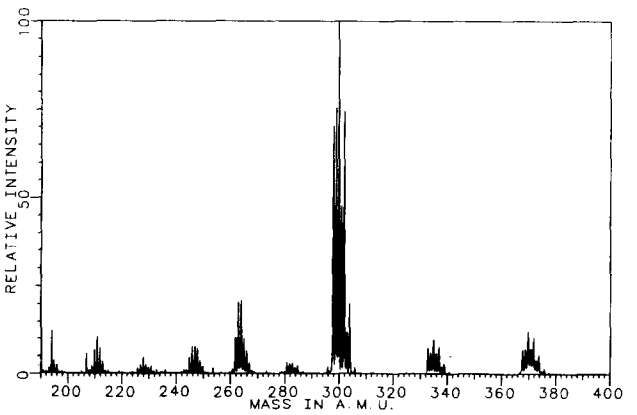

Figure 2
Positive ion spectrum of *cis*-dichlorodiammineplatinum(II) (Cisplatin) (see text for details).

ligands led to the observation of $[PtCl_2(NH_3)]^+$ (*m/z* 281), $[PtCl_2]^+$ (*m/z* 264), $[PtCl(NH_3)_2]^+$ (*m/z* 263), $[PtCl(NH_3)]^+$ (*m/z* 246), $[PtCl]^+$ (*m/z* 229), $[Pt(NH_3)_2]^+$ (*m/z* 228), $[Pt(NH_3)]^+$ (*m/z* 211) and Pt^+ (*m/z* 194). The clusters arise from the naturally occurring platinum isotopes (*m/z* 194, 195, 196 and 198) and chlorine isotopes (*m/z* 35 and 37). The *m/z* values listed above correspond to the lowest mass ion in each cluster. Also observed were clusters at *m/z* 333 and *m/z* 368, which correspond to the addition of Cl to form $[PtCl_3(NH_3)_2]^+$ and $[PtCl_4(NH_3)_2]^+$, respectively.

Figure 3 shows the high resolution positive ion mass spectrum of the parent ion cluster of cisplatin. The eight peaks expected are observed at *m/z* 298, 299, 300, 301, 302, 303, 304 and 306. The narrow peaks at *m/z* 303.1 and 305.4 correspond to noise spikes. The cisplatin peaks have the appropriate *m/z* values and appear in the expected ratios, corresponding to their natural abundances, with the exception of the peaks at *m/z* 301 and 302. No explanation for these discrepancies can be made at this time. Table 1 lists the calculated and observed relative abundances of the parent ion peaks. Table 1 also shows that three of the peaks are actually multiplet peaks, those at *m/z* 300 and 304 being doublets, while that at *m/z* 302 is a triplet. The largest separation of these multiplets, five milli-mass units, is too small to be observed at the resolution used to obtain this spectrum.

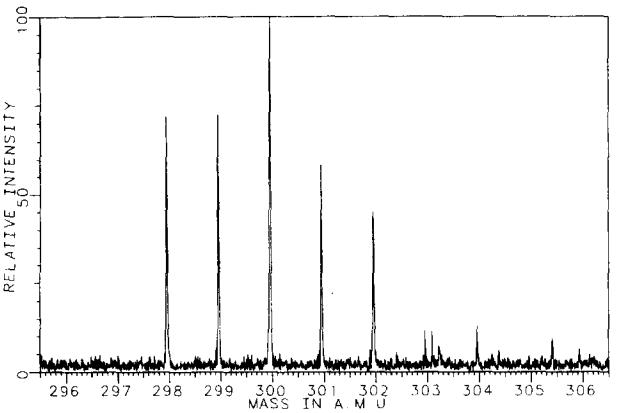

Figure 3
High resolution positive ion spectrum of the molecular ion cluster of *cis*-dichlorodiammineplatinum(II) (Cisplatin) (cf. Fig. 2).

The negative ion mass spectrum of cisplatin shows a cluster of peaks at *m/z* 264 (Fig. 4) and a minor cluster at *m/z* 281 ($[PtCl_2(NH_3)]^-$. The peak ratios of the cluster at *m/z* 264, together with the absence of a major peak at *m/z* 263, indicate that the cluster arises from $[PtCl_2]^-$ and not from $[PtCl(NH_3)_2]^-$. However, it should be noted that the minor peaks at *m/z* 262 and 263 could arise via loss of HCl from the parent ion.

Positive and negative ion spectra of *trans*-dichlorodiammineplatinum(II) were identical to those of the *cis*-isomer, except that they lacked the (M + Cl) and (M + 2Cl) clusters at short delay times.

Cis-dichlorocyclohexyl-1,2-diammineplatinum(II) did not afford as much information in its mass spectrum as did cisplatin. The positive ion spectra were very noisy and yielded little information in the *m/z* 180–450 range. The only peaks observed in this region were the *m/z* 264 cluster of $[PtCl_2]^+$, which were small compared with the peaks below *m/z* 100 arising from fragmentation of the organic ligand. The negative ion spectra also showed

Table 1
Calculated and observed peak intensities for the molecular ion cluster of the parent ion peak, $[PtCl_2(NH_3)_2]^+$

Cluster	*m/z*	Natural abundance (%)	Nominal *m/z*		Calculated relative peak height/area (%)	Observed relative peak height* (%)	Observed relative peak area* (%)
$^{194}Pt^{35}Cl^{35}Cl\ L_2$	297.95852	18.89	298		71	72	67
$^{195}Pt^{35}Cl^{35}Cl\ L_2$	298.96063	19.40	299		73	73	62
$^{194}Pt^{35}Cl^{37}Cl\ L_2$	299.95560	12.08	300	†	100	100	100
$^{196}Pt^{35}Cl^{35}Cl\ L_2$	299.96079	14.52	300				
$^{195}Pt^{35}Cl^{37}Cl\ L_2$	300.95771	12.41	301		47	59	47
$^{194}Pt^{37}Cl^{37}Cl\ L_2$	301.95262	1.93	302				
$^{196}Pt^{35}Cl^{37}Cl\ L_2$	301.95787	9.29	302	‡	58	46	43
$^{198}Pt^{35}Cl^{35}Cl\ L_2$	301.96372	4.13	302				
$^{195}Pt^{37}Cl^{37}Cl\ L_2$	302.95473	1.98	303		7	10	4
$^{196}Pt^{37}Cl^{37}Cl\ L_2$	303.95489	1.48	304	†	15	13	7
$^{198}Pt^{35}Cl^{37}Cl\ L_2$	303.96080	2.64	304				
$^{198}Pt^{37}Cl^{37}Cl\ L_2$	305.95782	0.42	306		2	5	1

* From spectrum in Fig. 3.
† Doublet.
‡ Triplet.

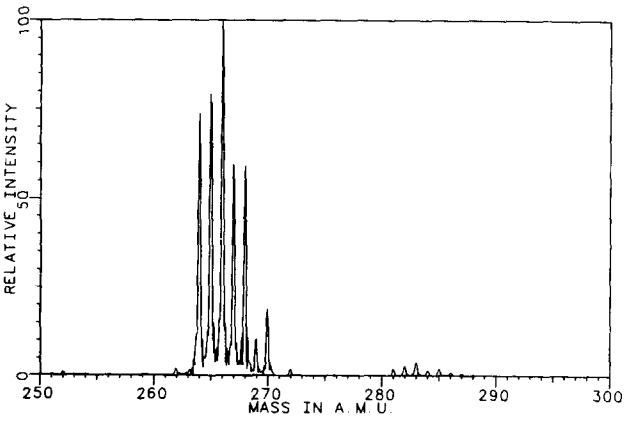

Figure 4
Negative ion spectrum of *cis*-dichlorodiammineplatinum(II) (Cisplatin) (see text for details).

the cluster of peaks at *m/z* 264 of $[PtCl_2]^-$. No parent ion cluster (*m/z* 378) was observed, but clusters at about *m/z* 282, 321 and 355 were present, probably attributable to ion–molecule reactions between $[PtCl_2]^-$ and various neutral species produced by the fragmentation of cyclohexyl-1,2-diamine.

The platinum(IV) complex, *cis*-dichloro-*trans*-dihydroxybis-2-propanamine-platinum(IV), CHIP, gave very complex positive ion spectra (Figs 5a and b). The six ligands give rise to a large number of isotopic clusters, due to their sequential loss, for which the *m/z* values are listed in Table 2. Most of the principal clusters were observed, except for some of the high mass clusters, including the parent ion cluster. The large

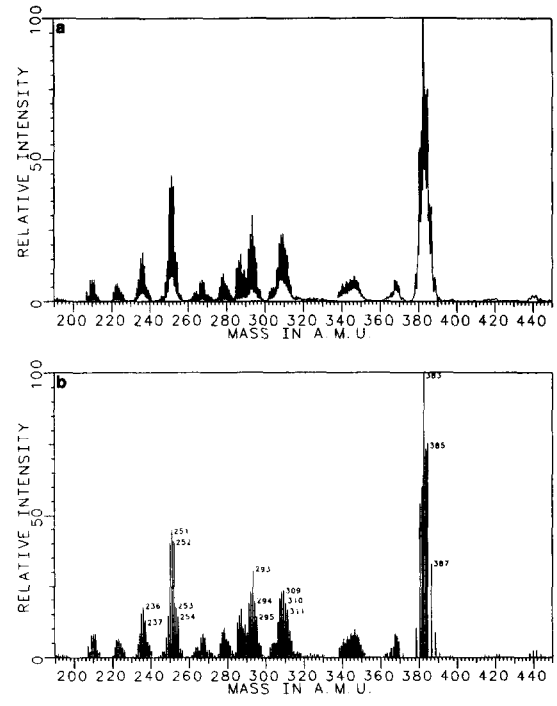

Figure 5
Positive ion spectrum of *cis*-dichloro-*trans*-dihydroxybis-2-propanamine-platinum(IV) (CHIP) (see Table 2 for assignation of peaks): (a) 'Magnitude mode' spectrum; (b) Resolved 'stick' spectrum of (a).

Table 2
Nominal *m/z* values of ion clusters formed from the sequential loss of ligands from CHIP. The lowest mass in the isotopic cluster is listed (cf. Fig. 5 for spectrum)

Nominal *m/z*	Ion	Nominal *m/z*	Ion
211	$Pt(OH)^+$	312	$Pt(C_3H_9N)_2^+$
*228	$Pt(OH)_2^+$	322	$Pt(C_3H_9N)(OH)_2Cl^+$
*229	$PtCl^+$	323	$Pt(C_3H_9N)Cl_2^+$
246	$Pt(OH)Cl^+$	329	$Pt(C_3H_9N)_2(OH)^+$
253	$Pt(C_3H_9N)^+$	340	$Pt(C_3H_9N)(OH)Cl_2^+$
263	$Pt(OH)_2Cl^+$	346	$Pt(C_3H_9N)_2(OH)_2^+$
264	$PtCl_2^+$	347	$Pt(C_3H_9N)_2Cl^+$
270	$Pt(C_3H_9N)(OH)^+$	*357	$Pt(C_3H_9N)(OH)_2Cl_2^+$
281	$Pt(OH)Cl_2^+$	364	$Pt(C_3H_9N)_2(OH)Cl^+$
287	$Pt(C_3H_9N)(OH)_2^+$	381	$Pt(C_3H_9N)_2(OH)_2Cl^+$
288	$Pt(C_3H_9N)Cl^+$	382	$Pt(C_3H_9N)_2Cl_2^+$
298	$Pt(OH)_2Cl_2^+$	*399	$Pt(C_3H_9N)_2(OH)Cl_2^+$
305	$Pt(C_3H_9N)(OH)Cl^+$	*416	$Pt(C_3H_9N)_2(OH)_2Cl_2^+$

* Not observed in the positive ion spectrum.

abundance of other ions may have obscured the signals from these clusters. It is possible that double resonance experiments may be found useful for investigating their existence. The negative ion spectra of CHIP primarily yielded a large $[PtCl_2]^-$ cluster and provided little additional information compared with the positive ion data.

Conclusions

In general, FT-MS can provide useful mass spectra of the cisplatin family of compounds. The main advantage of this technique compared with conventional mass spectrometry arises from the relatively few ions required for detection. The present results show that FT-MS data for platinum complexes can be obtained using standard techniques. FT-MS should provide an important tool in the analysis of model systems for biotransformation studies of the cisplatin compounds. The observation of the ion-molecule reaction products in both the cisplatin and the *cis*-dichlorocyclohexyl-1,2,diammineplatinum(II) complex indicates the existence of new reaction pathways. Double resonance experiments should be capable of characterizing these reactions.

Other techniques for volatilization and ionization should find use in the study of higher molecular weight complexes, both for the parent compound and for biotransformation products. Laser desorption and ionization are techniques which have been shown to be effective in the laser microprobe mass analysis (LAMMA) of platinum complexes [32]. However, the LAMMA spectra obtained by these workers [32] were of relatively poor quality compared with those presented here and appear to be useful only for the non-specific detection of platinum itself. The combination of laser desorption and ion cyclotron MS with FT data analysis [33] presents exciting possibilities for the future analysis of platinum complexes.

Acknowledgement: The purchase of the Nicolet FT/MS instrument was supported in part by the Department of Defense Research Instrumentation Program Contract No. N00014-83-6-0180.

References

[1] B. Rosenberg, E. Renshaw, L. Van Camp, J. Hartwick and J. Drobnick, *J. Bacteriol.* **93**, 716–721 (1967).
[2] A. Prestayko, S. T. Cooke and S. K. Carter (Eds), *Cisplatin: Current Status and New Developments.* Academic Press, New York (1980) and references therein.
[3] H. G. Bruckner, C. J. Cohen, R. C. Wallach, B. Kabakow, G. Deppe, E. M. Greenspan, S. G. Gusberg and J. F. Holland, *Cancer Treat. Rep.* **62**, 555–558 (1978).
[4] D. M. Hayes, E. Cvitkovic, R. B. Golbey, E. Scheiner, L. Helson and I. H. Krakoff, *Cancer* **39**, 1372–1381 (1977).
[5] C. E. Merrin, *Cancer Treat. Rep.* **63**, 1579–1584 (1979).
[6] D. D. Van Hoff and M. Rozencweig, *Adv. Pharmacol. Chemother.* **16**, 273–298 (1979).
[7] S. J. Lippard (Ed.) *Platinum, Gold and Other Metal Chemotherapeutic Agents; Chemistry and Biochemistry.* ACS Symposium Series 209, American Chemical Society, Washington, DC (1983) and references therein.
[8] R. J. Gralla, L. M. Itri, S. E. Pisko, A. E. Squillante, D. P. Kelsen, D. W. Braun, L. A. Bordin, T. J. Braun and C. W. Young, *New Eng. J. Med.* **305**, 905–909 (1981).
[9] C. L. Litterst, *Toxic. appl. Pharmac.* **61**, 99–108 (1981).
[10] A. P. Zipp and S. G. Zipp, *J. Chem. Educ.* **54**, 739–741 (1977).
[11] M. J. Cleare, *Platinum Metals Rev.* **18**, 122–129 (1974).
[12] M. J. Cleare and J. D. Hoeschele, *Platinum Metals Rev.* **17**, 2–13 (1973).
[13] P. D. Braddock, T. A. Connors, M. Jones, A. R. Khokhar, R. H. Melzack and M. J. Tobe, *Chem. Biol. Interact.* **11**, 145–161 (1975).
[14] J. J. Roberts and M. F. Pera, Jr., in *Platinum, Gold and Other Metal Chemotherapeutic Agents; Chemistry and Biochemistry* (S. J. Lippard, Ed.), pp. 3–25. ACS Symposium Series 209; American Chemical Society, Washington, DC (1983).

[15] M. J. Cleare, P. C. Hydes, B. W. Malerbi and D. M. Watkins, *Biochimie* **60**, 835–850 (1978).
[16] C. M. Riley, L. A. Sternson, A. J. Repta and S. A. Slyter, *Polyhedron* **1**, 201–202 (1982).
[17] S. J. Lippard, *Science* **218**, 1075–1082 (1982).
[18] C. M. Riley, L. A. Sternson, A. J. Repta and S. A. Slyter, *Anal. Biochem.* **130**, 203–214 (1983).
[19] S. Mansy, B. Rosenberg and A. J. Thomson, *J. Am. Chem. Soc.* **95**, 1633–1640 (1973).
[20] P. Horack and J. Drobnik, *Biochim. Biophys. Acta* **254**, 341–347 (1971).
[21] N. P. Johnson, J. D. Hoeschele and R. O. Rahn, *Chem. Biol. Interact.* **30**, 151–169 (1980).
[22] M. Nee and J. D. Roberts, *Biochemistry* **21**, 4920–4926 (1982).
[23] C. M. Riley, L. A. Sternson and A. J. Repta, *Anal. Biochem.* **124**, 167–179 (1982).
[24] A. F. LeRoy, M. Wehling, P. Gromley, M. Egorin, S. Ostrow, N. Bachur and P. Wiernik, *Cancer Treat. Rep.* **64**, 123–132 (1980).
[25] A. J. Repta and D. F. Long, in *Cisplatin: Current Status and New Developments* (A. Prestayko, S. T. Cooke and S. K. Carter, Eds), Chapter 18, pp. 285–304. Academic Press, New York (1980).
[26] K. J. Himmelstein, T. F. Patton, R. J. Belt, S. Taylor, A. J. Repta and L. A. Sternson, *Clin. Pharmacol. Ther.* **29**, 658–664 (1981).
[27] T. S. Gill, D. K. Luscombe and R. G. Fish, *J. Pharm. Pharmacol.* **33**, 44P (1981).
[28] C. M. Riley, L. A. Sternson, A. J. Repta and R. W. Siegler, *J. Chromatogr.* **229**, 373–386 (1982).
[29] M. B. Comisarow and A. G. Marshall, *Chem. Phys. Lett.* **25**, 282–283 (1974).
[30] C. L. Wilkins, *Anal. Chem.* **50**, 493A–500A (1978).
[31] C. L. Wilkins and M. L. Gross, *Anal. Chem.* **53**, 1661A–1676A (1981).
[32] A. H. Verbueken, R. E. Van Grieken, G. J. Paulus, G. A. Verpooten and M. E. De Broe, *Biomed. Mass Spectrom.* **11**, 159–163 (1984).
[33] D. A. McCrery, E. B. Ledford, Jr. and M. L. Gross, *Anal. Chem.* **54**, 1435–1437 (1982).

[*Received for review 26 September 1984*]