ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
20 (1999) 115–128

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Monitoring in vitro experiments using microdialysis sampling on-line with mass spectrometry

Edward H. Kerns [1], Kevin J. Volk *, Steven E. Klohr, Mike S. Lee[1]

Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492, USA

Received in revised form 18 December 1998; accepted 7 January 1999

Abstract

A method has been developed for the real-time analysis of components in in vitro reactions by the on-line combination of microdialysis sampling (MD) with tandem mass spectrometry (MS/MS) and single stage mass spectrometry (MS). Apparatus and parameters associated with the integration have been studied. Analytical figures of merit for the drug gepirone have been determined. The qualitative 'limit of identification' was found to be 100 ng/ml and 200 ng/ml for methods using thermospray and electrospray MS interfaces, respectively. Using this approach, monitoring of in vitro experiments involving drug metabolites, enzymatic reactions, and ligand-protein binding interactions were performed. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Enzyme reaction monitoring; Metabolite; Microdialysis; Protein binding; Tandem mass spectrometry

1. Introduction

Analytical strategies for mixture analysis have commonly required incorporation of sample preparation steps for scale-up, extraction, fractionation, storage and multiple instrumental analysis of each fraction. These steps can be time-consuming, thus slowing progress and, sometimes, leading to component degradation. In order to reduce analysis time and increase efficiency analytical methodologies are sometimes integrated into one instrumental unit. Examples include the on-line combination of gas chromatography and mass spectrometry [1], two-dimensional column switching chromatography methods [2] and tandem mass spectrometry (MS/MS) [3–5]. Previously, MS/MS methods have been developed for the rapid identification of trace components in a wide variety of complex mixtures with minimal sample preparation, such as drug metabolites in physiological matrices [6–9], drug impurities [10], degradants [11] in bulk or formulated drug and natural products [12–14] in crude extracts.

* Corresponding author. Present address: Bristol-Myers Squibb Pharmaceutical Research Institute, Rt. 206 & Province Line Road, Princeton, NJ 08543-4000, USA. Tel.: +1-609-252-3711.

[1] Present address: Milestone Development Services, P.O. Box 813, Pennington, NJ 08534-0813, USA.

0731-7085/99/$ - see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0731-7085(99)00003-5

In order to reduce sample storage and preparation steps, we have investigated the integration of microdialysis (MD) sampling with MS/MS. MD is a sampling technique which incorporates a dialysis membrane into a microprobe (0.4 mm ID) that can be inserted directly into a sample. MD was developed for in vivo sampling of brain neurotransmitters from living animals [15–20] and its applications have extended into other areas of in vivo biomedical research, such as pharmacokinetics [21–24]. A unique feature of the MD technique is that it permits continuous sampling while producing minimal disruption of the sample's chemical balance. This is possible because the dialysis membrane (5–30 kDa cut-off) maintains the fluid and protein concentration of the sample. The integration of MD on-line with other analytical techniques, such as HPLC [25,26], has increased sample throughput and experimental efficiency. MD has also been coupled with MS/MS via a continuous-flow fast atom bombardment interface for on-line quantitative analysis of penicillins G and V, cocaine, benzoyl ecgonine, valproic acid and tris(2-chloroethyl) phosphate and the identification of substance P metabolites in living animals [27–34].

Here we describe the results of studies dealing with integration of MD, for in vitro sampling, with MS and MS/MS via commonly available thermospray and electrospray MS interfaces. Methods for each interface were optimized for sampling time, dialysis fluid flow rate, analyte transit time and MD sampling efficiency. Optimum conditions were investigated using MS/MS selected reaction monitoring (SRM) response. The performance of each system was evaluated for linearity, limit of detection (LOD) and limit of identification [1] (LOI) using the CNS drug gepirone as a model. The systems were subsequently applied for on-line analyses involving drug metabolite screening. The utility of MD for on-line monitoring of enzymatic reactions and molecular interaction studies was further investigated using single stage MS analysis.

2. Experimental

2.1. Materials

Ammonium acetate and glacial acetic acid were obtained from Fisher (Fairlawn, NJ, USA). Am-

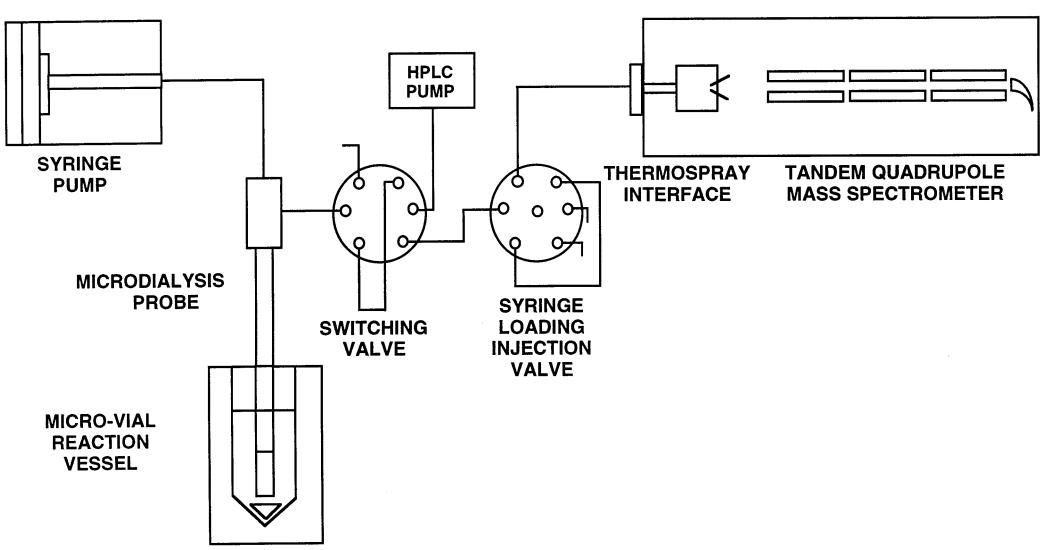


Fig. 1. Apparatus design for the integration of microdialysis sampling with thermospray MS/MS (components not to scale). Details are provided in the Section 2. The integrated electrospray system was similar, except that MD dialysate was allowed to flow directly to the electrospray interface without intervening valves.

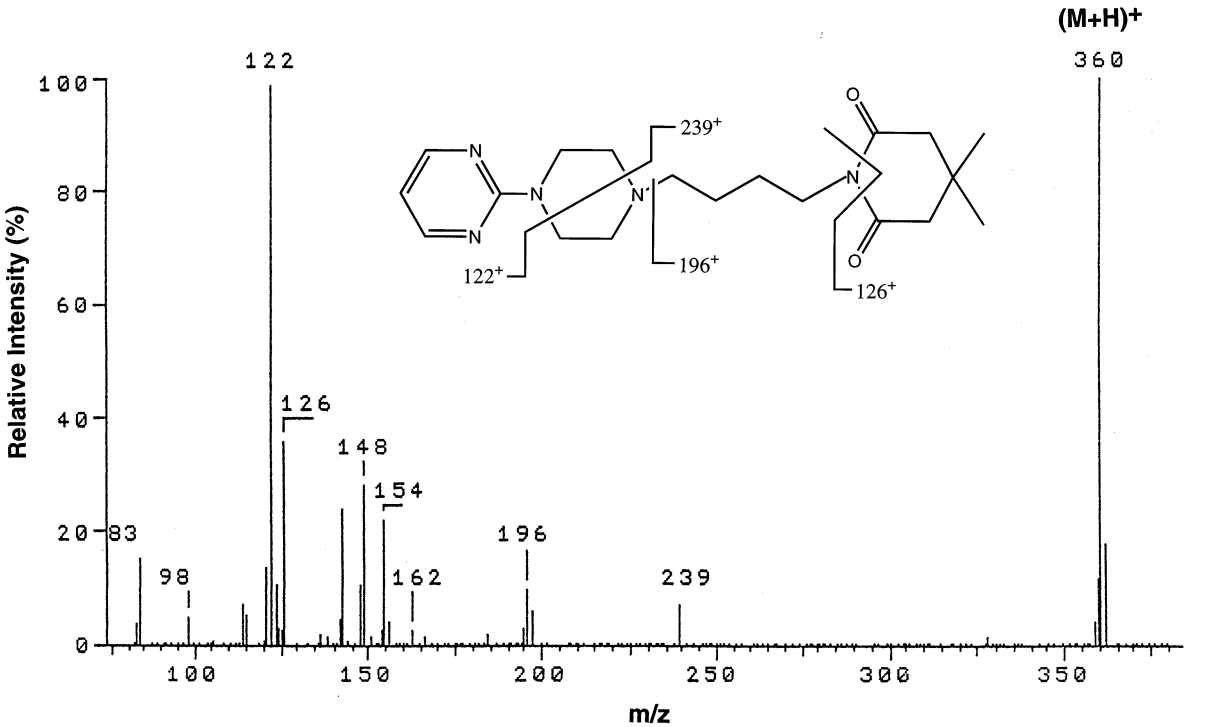


Fig. 2. The structure and thermospray MS/MS product ion spectrum of gepirone. The CID transition of the m/z 360 $(M+H)^+$ ion to the m/z 122 product ion was monitored using SRM for quantitative studies. This spectrum was obtained on-line from MD sampling of a 500-ng/ml gepirone solution in 50 mM ammonium acetate buffer.

monium acetate was dissolved at 2 or 50 mM (pH 6.8) in double-distilled water from an in-house distillation system. Glacial acetic acid was dissolved to 0.05% (v/v) in double-distilled water. Gepirone, 3-hydroxy gepirone, and 1-pyrimidinyl piperazine were synthesized in-house. Substance P, [Lys^3]bombesin, carboxypeptidase Y, avidin, and biotin methyl ester (BME) were obtained from Sigma (St. Louis, MO, USA). Rat plasma was obtained from Hilltop Lab Animals (Scottsdale, PA, USA).

2.2. Instrumentation

2.2.1. Thermospray studies

A Finnigan MAT (San Jose, CA, USA) TSQ70 tandem quadrupole mass spectrometer equipped with a Vestec (PerSeptive Biosystems, Cambridge MA, USA) 704A thermospray interface was utilized. The integrated apparatus is shown in Fig. 1. A Bioanalytical Systems (West Lafayette, IN, USA) Carnegie-Medicin CMA/100 syringe pump supplied dialysis fluid (50 mM ammonium acetate) at 10 μl/min (except where noted) to a CMA/10 microdialysis probe (20 × 0.4 mm ID, 4 mm membrane). A flow rate of 10 μl/min is higher that typically used for microdialysis studies; however, this flow rate reduced the fluid transit time to the thermospray MS system and dilution due to diffusion in the transit tubing. The probe was immersed in the aqueous in vitro sample in a 0.5-ml Pierce Reacti-Vial stirred with a magnetic stirrer at 37°C by a Pierce (Rockford, IL, USA) Reacti-Therm Heating/Stirring Module. The probe was connected to a Rheodyne (Cotati, CA, USA) 7000RV switching valve (SV). This connection utilized a 200 × 0.12 mm ID PTFE tube and a 50 mm × 0.005″ ID SS tube joined by a 1/16″ to 1/32″ reducing union. Dialysate was captured in the 50-μl sample loop of the SV for 1.0 min and then back-flushed from the loop using 50 mM ammonium acetate at 1.5 ml/min. The SV was

recycled to the sample collection position after 10 s in the back-flush position. The SV was connected to a Rheodyne 7125 syringe loading injection valve (SLIV) via a 200 mm × 0.010″ ID SS tube. A 100-μl sample loop on the SLIV permitted the direct injection of calibration standards into the MS/MS instrument. The SLIV was connected to the thermospray interface via a 500 mm × 0.015″ ID SS tube. MS/MS experiments were conducted with 0.7 mTorr argon collision gas and 50 eV collision energy.

2.2.2. Electrospray studies

A PE SCIEX (Concord, Ont., Canada) API III tandem quadrupole mass spectrometer equipped with an Ionspray® (nebulizer-assisted electrospray) interface was utilized. The apparatus was similar to that shown in Fig. 1, except that the MD probe was connected directly to the electrospray interface without intervening valves. This connection utilized a 200 × 0.12-mm ID PTFE tube connected to the 100 mm × 97 μm ID fused-silica tube of the interface via a vespel butt union secured with epoxy cement. The CMA/100 syringe pump supplied dialysis fluid at 5 μl/min, except where noted. Dialysis fluid was 0.1% acetic acid for analysis of peptides and 2 mM ammonium acetate for other studies. The MD probe was grounded to the MS/MS chassis. Full scan spectra were obtained with a scan time of 2 s. MS/MS experiments were conducted with 450×10^{12} atoms/cm^2 argon curtain gas and 50 eV collision energy.

2.3. Methods

2.3.1. Thermospray method development studies

Evaluation of MD sampling with the thermospray interface was conducted to determine the optimum operating conditions, sensitivity, and linearity using the CNS drug gepirone, spiked into rat plasma. The gepirone quantitative response was monitored using selected reaction monitoring MS/MS (SRM) for the ($360^+ \rightarrow 122^+$) decomposition at a scan rate of 0.5 s/scan without prior separation from other sample components.

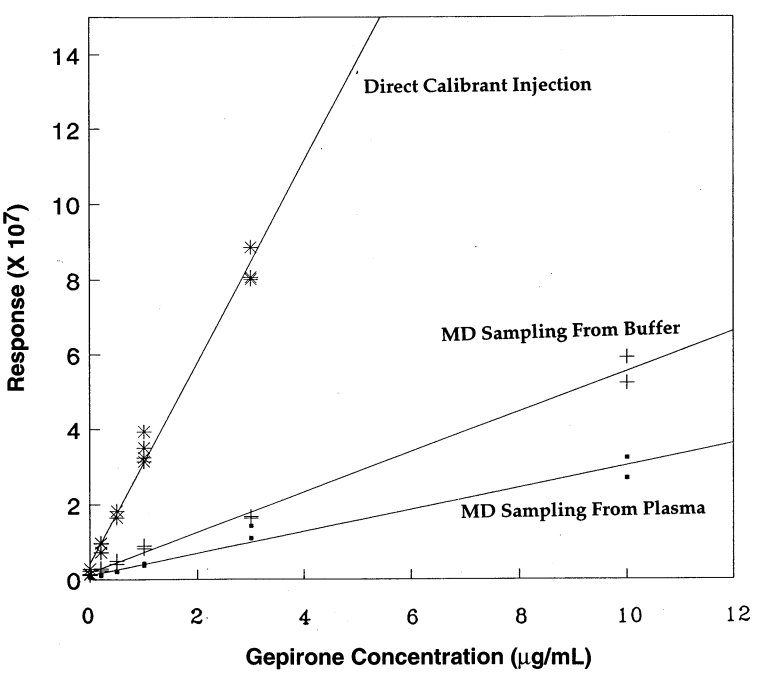


Fig. 3. Response of the integrated thermospray system for gepirone directly injected via the SLIV, MD sampled from buffer (50 mM ammonium acetate) and MD sampled from rat plasma.

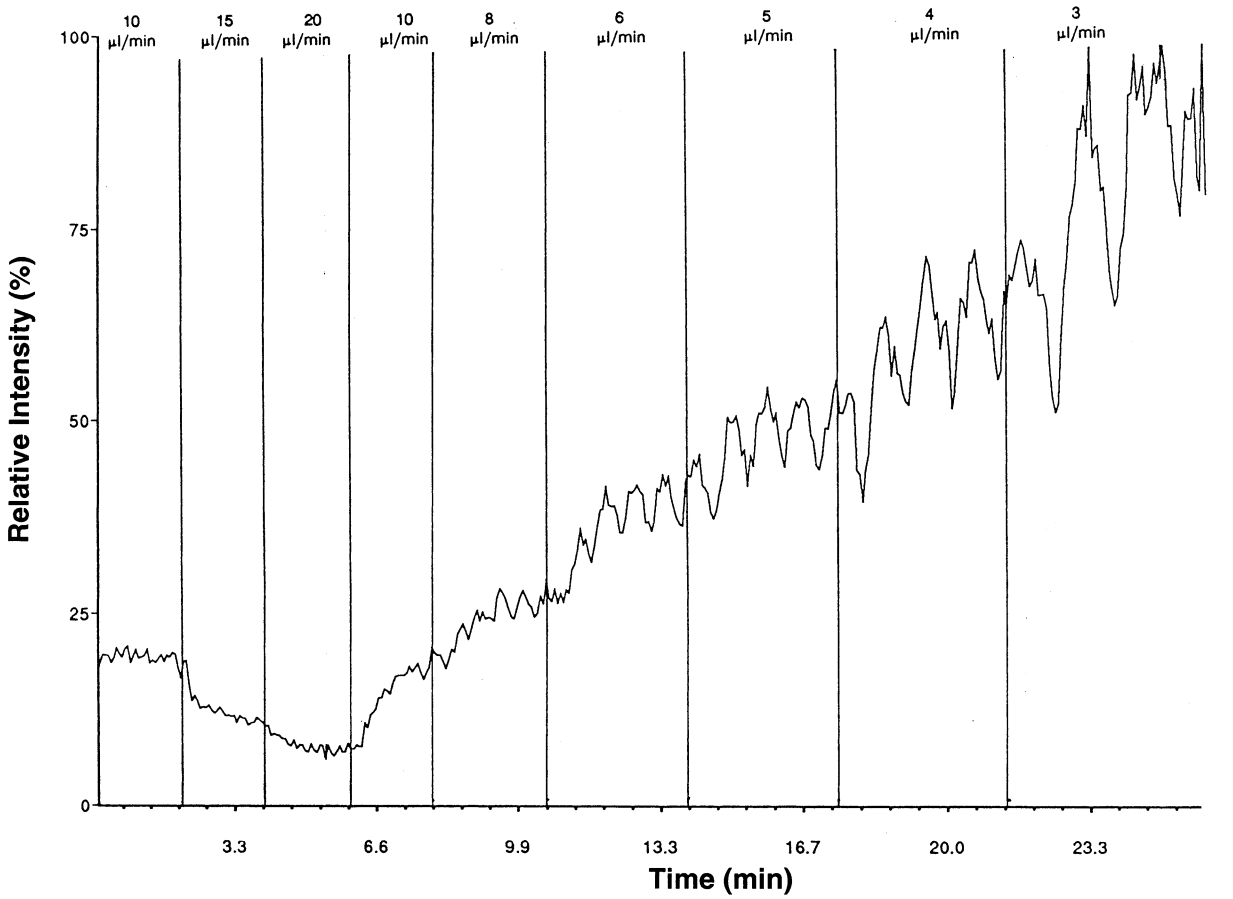


Fig. 4. Response of the electrospray system at m/z 360 of gepirone sampled from rat plasma at various dialysis flow rates ranging from 3 to 20 µl/min.

The relationship between sampling time and quantitative response was investigated by analyzing dialysate collected in the valve loop for 1, 2 and 4 min. The transit time of analyte through the system to the mass spectrometer was measured from the time the microdialysis probe was placed into the solution until the time the analyte was detected at the mass spectrometer, with dialysate SV capture time reduced from 1.0 to 0.5 min. The LOD and linearity were evaluated by dialyzing solutions of gepirone spiked into rat plasma over the concentration range of 0.02–10 µg/ml. The LOD was the concentration at which a response of three times the standard deviation of background signal variation from blank rat plasma was obtained. The effect of dialysis flow rate on sensitivity was studied by placing the MD probe in the sample, setting the dialysis flow rate from 5 to 50 µl/min, perfusing for at least 10 min with the SV in the waste position, then switching the SV to collect dialysate for 1.0 min, and subsequently sweeping the collected dialysate into the MS/MS. The efficiency of gepirone MD sampling from solution was measured at a dialysis flow rate of 10 µl/min by comparing the system responses of gepirone dialyzed from solution (50 mM ammonium acetate buffer and rat plasma) to a gepirone standard (in 50 mM ammonium acetate) injected directly into the mass spectrometer via the SLIV. The LOI [1] was determined by operating at successively lower concentrations of gepirone in plasma with 5.0 min sampling time.

The MS/MS product ion spectra of the gepirone $(M+H)^+$ ion were examined for the presence of ions structurally diagnostic of gepirone.

2.3.2. Electrospray method development studies

Evaluation of MD sampling in combination with electrospray also utilized gepirone. The transit time, LOD, LOI, and effect of dialysis flow rate on system response were measured in the same manner as described for the thermospray experiments, except that no sampling valve was used and the dialysate was allowed to flow directly into the electrospray interface.

2.3.3. In vitro analyses

MS/MS screening of drug metabolites in plasma was conducted using the integrated MD/MS/MS system. Rat plasma spiked at 500 ng/ml with gepirone and its metabolites 1-pyrimidinyl piperazine and 3-hydroxy gepirone was sampled using MD. MS/MS precursor ion scans were per formed, using the electrospray interface, directly on the dialysate fluid for precursors of m/z 122, which is diagnostic for metabolites containing the pyrimidinyl piperazine substructure of gepirone. Subsequent MS/MS product ion analysis of the $(M+H)^+$ ions detected from the precursor screen provided substructural conformation of the metabolite structures [1,2].

Monitoring of substance P and [Lys^3]bombesin enzymatic digestion by carboxypeptidase Y^{35} was performed on peptides dissolved at a concentration of 80 ng/μl in buffer (50 mM ammonium acetate, pH 6.0) to a final volume of 0.5 ml. Peptide cleavage reactions were performed at 25°C. Reactions were initiated by addition of

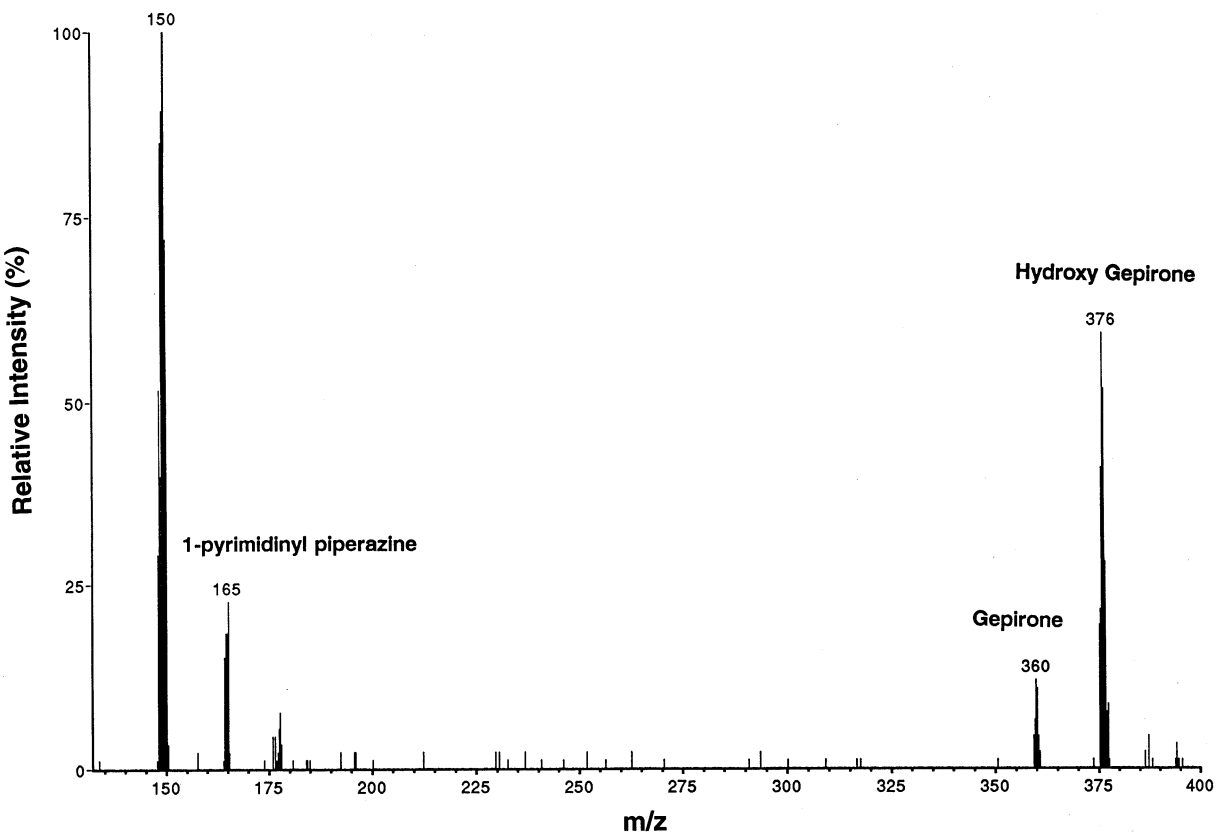


Fig. 5. MS/MS precursor (m/z 122) screen, diagnostic of the pyrimidinyl piperazine substructure, in rat plasma having gepirone and metabolites.

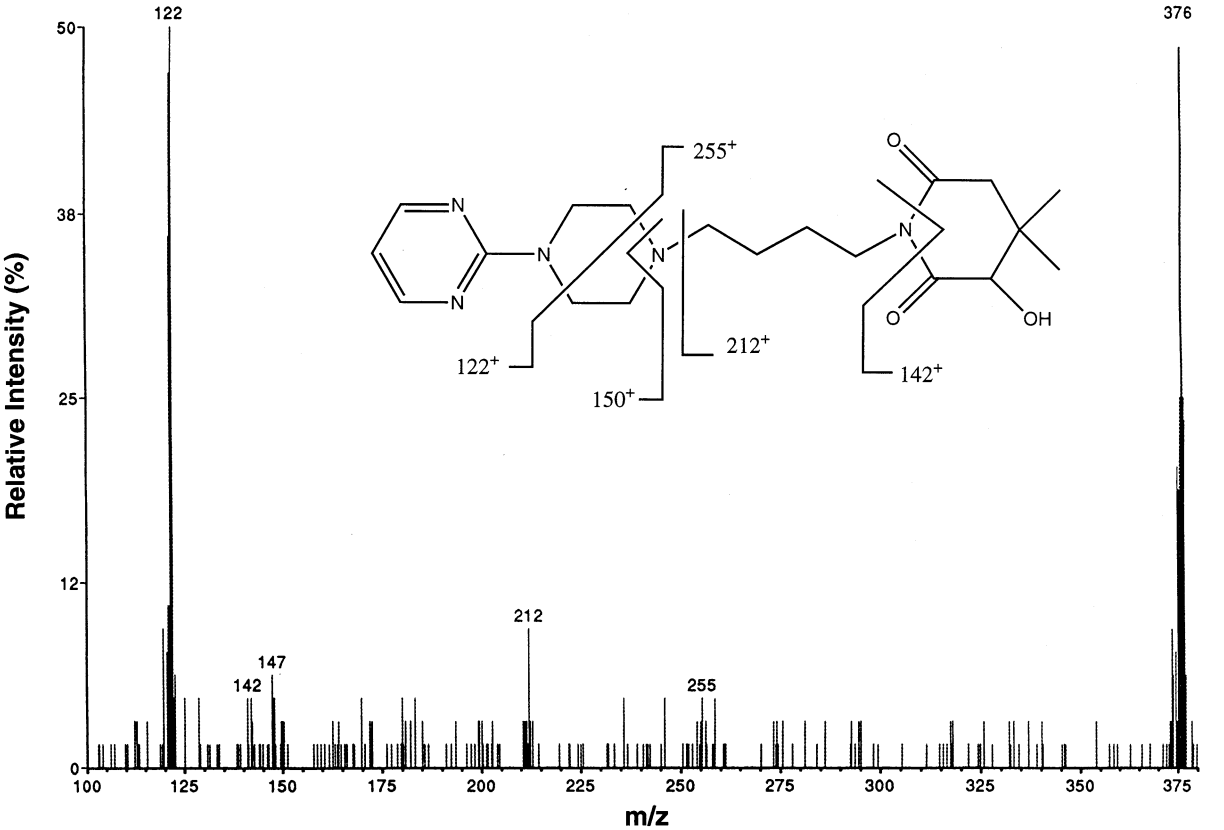


Fig. 6. MS/MS product ion spectrum of m/z 376 for 3-hydroxy gepirone analyzed on-line from rat plasma using MD and the integrated electrospray system.

carboxypeptidase Y to the buffered peptide solution. Full scan electrospray mass spectra of the dialysate fluid were continuously acquired for the dialysate routed directly to the electrospray interface.

Studies of the in vitro binding of BME to avidin in 2 mM ammonium acetate buffer utilized the electrospray system. Successive additions of 20 μmol of BME were followed by successive additions of 1.0 unit of avidin (defined as the amount avidin required to bind 20 μmol of biotin). The MD membrane blocked diffusion of avidin and avidin–BME complex and allowed only unbound BME in solution to be captured in the dialysis fluid. Full scan spectra were obtained from m/z 150–400 each 2 s. The presence of BME was detected by its characteristic $(M + H)^+$ and $(M + NH_4)^+$ ion signals at m/z 259 and m/z 276, respectively.

3. Results and discussion

3.1. Thermospray method development studies

The structure and thermospray MS/MS product ion spectrum of the CNS drug gepirone are shown in Fig. 2. This spectrum was obtained from MD sampling of gepirone at 500 ng/ml in 50 mM ammonium acetate buffer solution. The collision induced decomposition (CID) ions are characteristic of specific substructures of the gepirone molecule, such as the m/z 122 ion for the pyrimidinyl piperazine substructure. This fragmentation pattern formed the basis for subsequent MS/MS product, precursor and selected reaction monitoring experiments.

Sample agitation during MD sampling was observed to have a significant effect on the intensity

of the gepirone MS response. Agitation of the solution surrounding the MD probe via a stirring bar or manual manipulation of the vial produced approximately 80% greater responses than MD sampling of a static solution. Thus, the concentration of gepirone in the dialysis fluid was increased when the sample was stirred. It is likely that fluid movement presents a continuously refreshed sample to the MD probe compared to a concentration gradient established in static solutions. Agitation is, therefore, necessary to obtain homogeneous MD sampling of in vitro experiments and was used in subsequent MD experiments.

Sampling time and response were found to be directly related from 1 to 4 min. This result indicates that in dynamic in vitro experiments different sampling times may be used at different time points and a correction factor for sampling time may be applied. This approach would be useful for experiments in which sampling times may be reduced when the analyte concentration is high and may be increased to enhance sensitivity when the analyte concentration is low. A sampling time of 1.0 min is used in subsequent experiments.

A time delay results from the transport of dialysate from the MD probe to the MS interface. Under the configuration in Fig. 1 gepirone was first observed in the 0.5-min sample collected from 2.0 to 2.5 min, indicating a transit time of 2.0–2.5 min. Based on a flow rate of 10 μl/min, the dead volume of the system is apparently 20–25 μl. It was observed to decrease as the internal volume of connecting tubing, microdialysis probe and valves were decreased. Transit time would have to be accounted for in experiments dealing with dynamic in vitro processes.

The LOD was determined to be 20 ng/ml of gepirone in rat plasma with a 1.0-min sampling time at 10 μl/min dialysis flow rate. The response

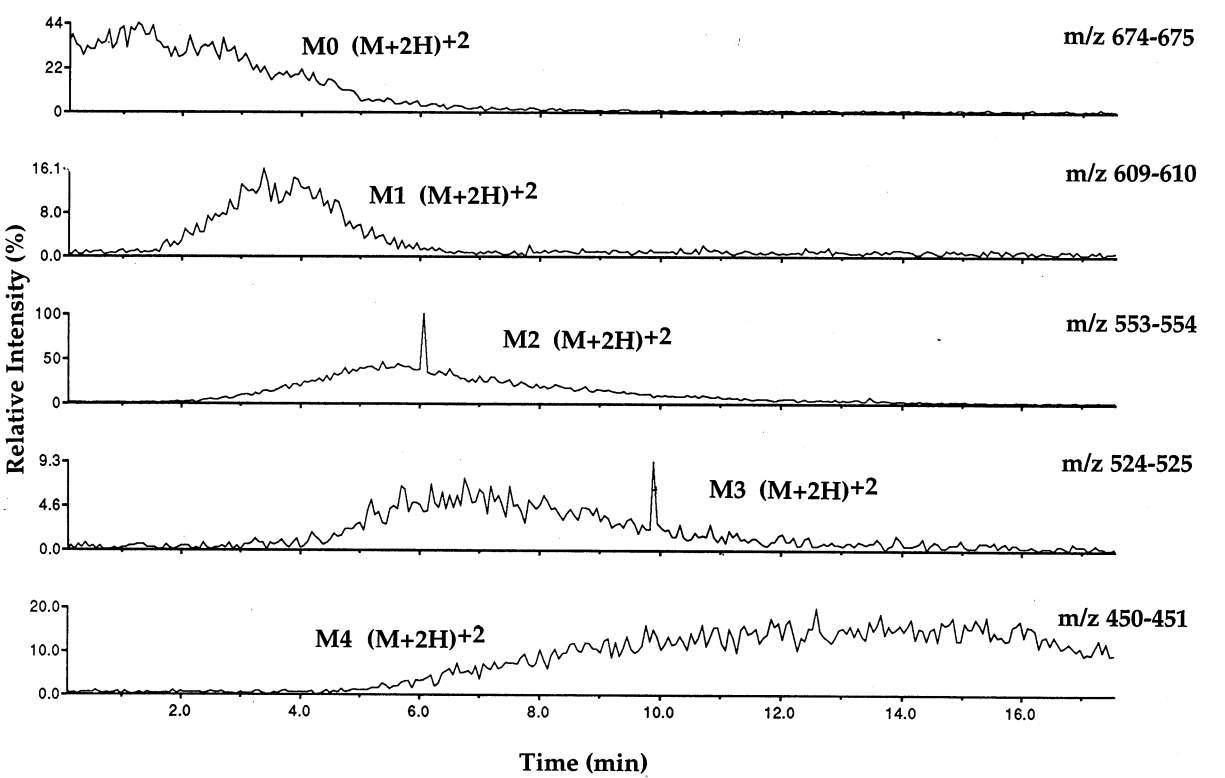


Fig. 7. Enzymatic reaction monitoring for the carboxypeptidase Y cleavage of substance P. Appearance and disappearance of sequence-indicating peptide reaction products, monitored using the integrated electrospray system.

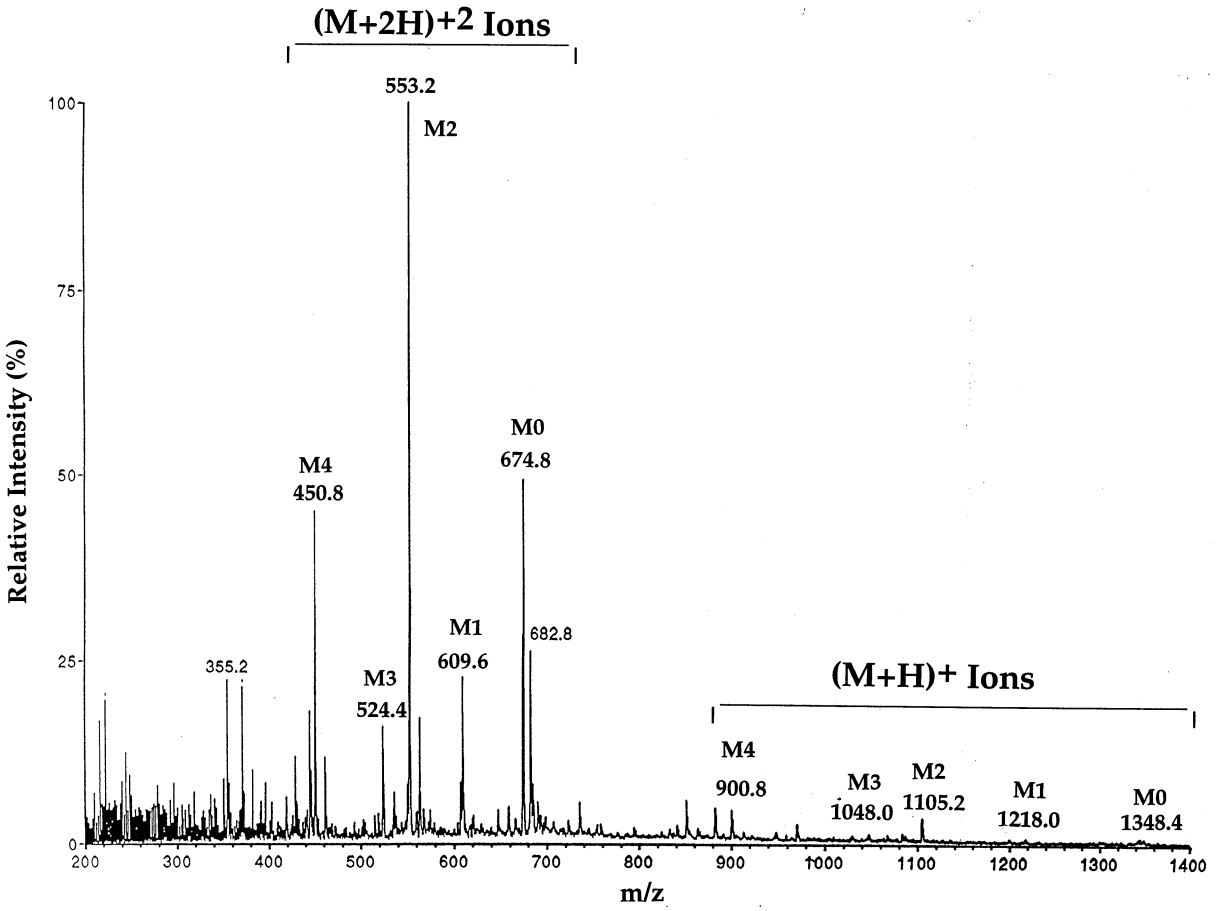


Fig. 8. The full scan mass spectra summed over 0–20 min from monitoring the carboxypeptidase Y cleavage of substance P. A profile of the molecular weights of the sequence specific substance P peptide cleavage products was observed.

of the system was found to be linear from 0.02 to 10 μg/ml (Fig. 3). This concentration range is consistent with drug concentrations utilized in drug discovery research.

Dialysis flow rate had no measurable effect on system response. Care was taken to assure that all of the collected sample was transferred into the ion source by perfusing at the desired flow rate for 10 min to assure that the MD probe and connecting tubing were filled with dialysate. At 50 μl/min the 1.0-min sample completely filled the sampling loop. These results were consistent with a linear rate of analyte transfer across the MD membrane into the dialysis fluid. (In many MD applications the flow rate is kept as low as possible to maximize analyte concentration in the collected sample. However, in the present studies the entire sample collected over a period of time is transferred to the MS detector in a narrow band.) A flow rate of 10 μl/min was used in subsequent thermospray experiments.

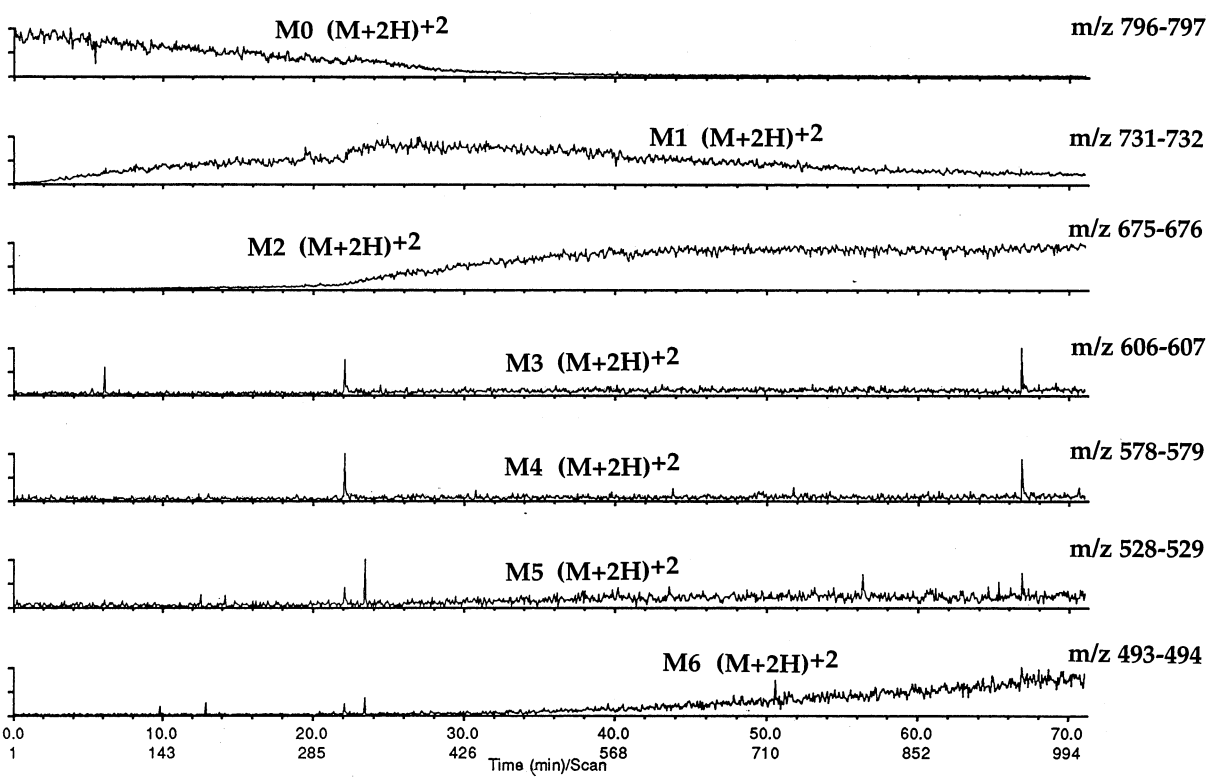
The efficiency for sampling of gepirone was found to be 20% from 50 mM ammonium acetate solution and 12% from rat plasma (Fig. 3), compared to standards injected directly into the mass spectrometer for calibration. The 40% lower response from plasma may be due, in part, to binding of gepirone to plasma protein as has been previously discussed [27]. This capability should be investigated for the rapid, semi-quantitative assessment of plasma protein binding.

The LOI was found to be 100 ng/ml. This analytical figure of merit indicates the lowest concentration at which diagnostic MS/MS product ions of gepirone could be observed for structure elucidation purposes. This result indicated that the thermospray system provides sufficient sensitivity for the concentrations of metabolites commonly encountered in MS/MS drug metabolite screening experiments.

The dual valves of the thermospray system serve several functions. They offer an external means of calibration by injection of standards (via the SLIV), protect the pressure-sensitive MD membrane from the high pressure (approximately 1000 psi) of the thermospray interface (via the SV) and separate the subsystems for independent optimization of conditions for MD sampling and ionization (e.g. flow rate, fluid composition). Flexibility is afforded to alter conditions depending on the physical characteristics of the compound or the in vitro experiment being studied. Maximum sensitivity is obtained from the narrow band of analyte produced when the dialysate in the sampling loop is rapidly swept into the thermospray interface. Ionization-promoting mobile phase components are mixed with the dialysate, providing optimum thermospray sensitivity despite dialysis fluid composition. The rapid back-flush sweep of the 10 μl dialysate sample from the sampling loop into the thermospray interface by the 1.5-ml/min thermospray mobile phase permits rapid recycling of the SV to the sampling position. This permits fast sequencing between sampling and analysis for maximum sample collection and short sampling intervals.

3.2. Electrospray method development studies

MD sampling with the electrospray interface offers several complementary characteristics to



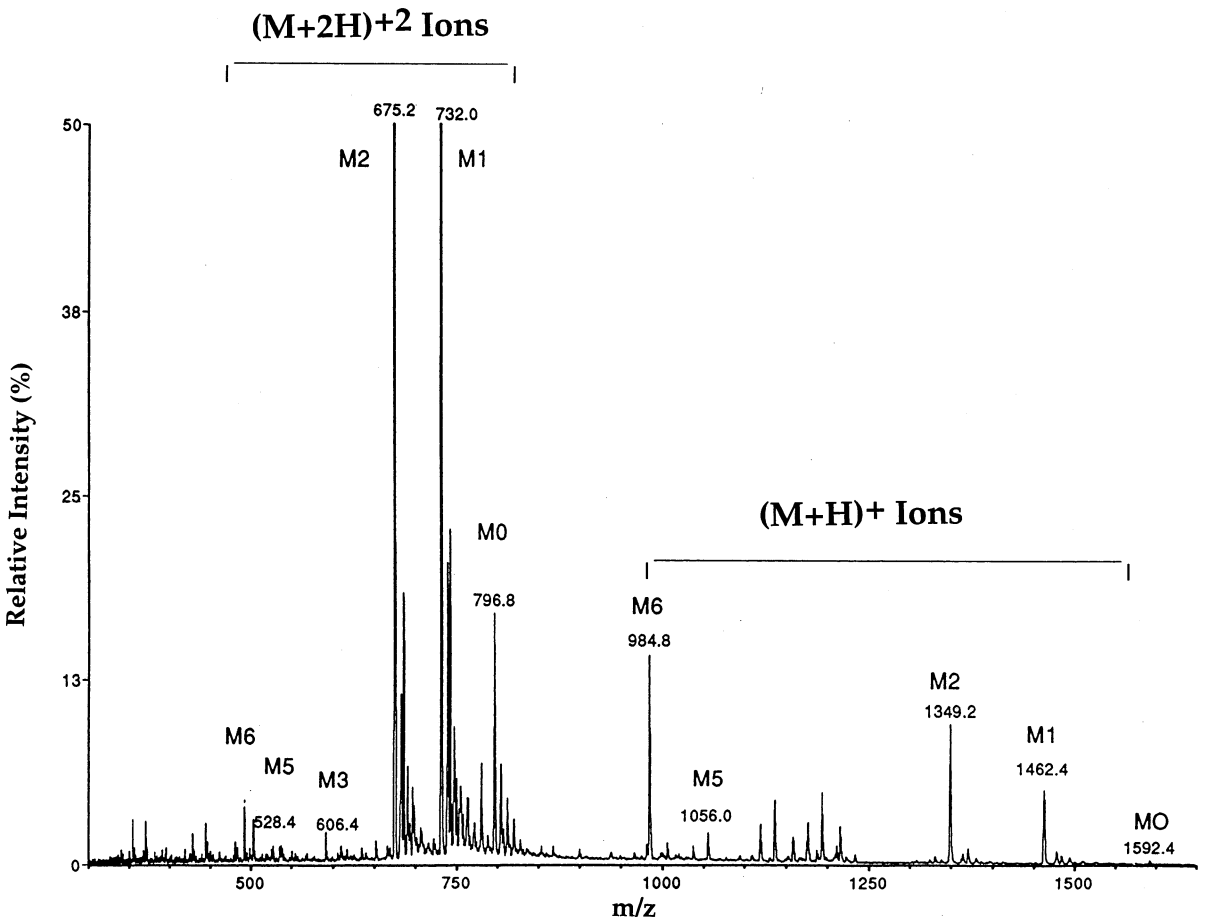


Fig. 10. The full scan mass spectra summed over 0–20 min from monitoring the carboxypeptidase Y cleavage of [Lys^3]bombesin. A profile of the molecular weights of the sequence specific [Lys^3]bombesin peptide cleavage products was observed.

the thermospray interface. First, electrospray operates at much lower back pressure than thermospray. This allows the pressure-sensitive dialysis membrane to be on-line with the electrospray interface without an intervening pressure neutralization valve. Second, electrospray can also operate at low flow rates (μl/min) commonly utilized for MD sampling. Finally, electrospray is very sensitive, making it compatible with the trace analytes common in MD dialysates.

A possible disadvantage of electrospray with MD sampling would occur with interference and performance degradation from high salt physiological buffers (e.g. Ringers) used in many MD experiments. Other investigators have used a reverse-phase trap [35] or column with divert valve [36] to reduce salts. The experiments described here have focused on in vitro experiments that do not require such buffers. Volatile buffers, compatible with mass spectrometry allow direct analysis of dialysate from in vitro experiments to flow directly into the electrospray interface for rapid analysis. Such experiments include the screening of physiological fluids and the sequencing and substructural analysis of molecules of biological interest. Thus, MD may be used as a rapid sample preparation method for in vitro, experiments in combination with the powerful separation and analysis capabilities of MS/MS to extend analysis protocols.

The effect of dialysis flow rate on electrospray response is shown in Fig. 4. Response increased proportionately as the dialysis flow rate was reduced. Optimum sensitivity was obtained at flow rates in the low μl/min range. This observation is consistent with the increased residence time of the dialysis fluid in the probe exposed to the sample as flow rate is reduced. It is well known that dialysis efficiency increases as dialysate flow rate decreases, resulting in increased concentration of analyte in the dialysate. Electrospray is a concentration-dependent ionization method producing greater ion yields as the concentration of analyte increases. Thus, low flow rate is favorable for optimum sensitivity of both the electrospray interface and MD sampling. The cyclic variation in signal seen in Fig. 4 likely results from small variations in the pressure generated by the syringe pump, which would affect the electrospray sensitivity. A constant pressure pumping device may be useful in reducing this variation.

The transit time of analyte through the system was found to be 2.0 min, indicating a dead volume of about 10 μl. The LOD is 100 ng/ml and the LOI was found to be 200 ng/ml for gepirone in rat plasma, which is adequate for analyte concentrations often found in in vitro experiments.

Use of the electrospray interface provides continuous sampling of the dialysate compared to the 1.0-min time points necessitated by valve sampling with the thermospray system. This system can, therefore, provide increased temporal resolution for dynamic chemical processes compared to MD experiments utilizing sample collection at time-points from 1 to 30 min. Acquisition of a continuous data set can also provide flexibility in

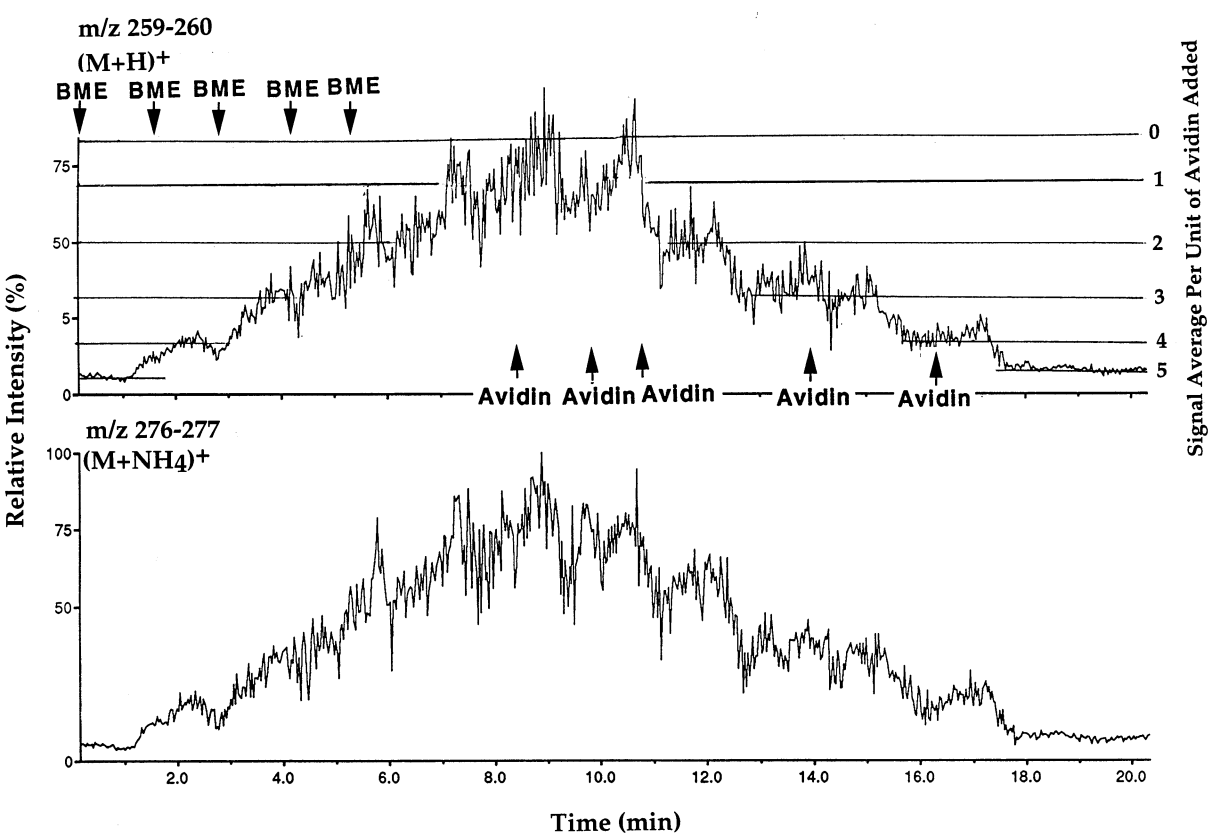


Fig. 11. The $(M+H)^+$ and $(M+NH_4)^+$ ion signals for BME resulting from sequential 20-μmol additions of BME to buffer, followed by sequential additions of 1.0 unit of avidin, monitored using the integrated electrospray system.

mass spectral data analysis for S/N enhancement, such as spectral averaging, spectral addition, background subtraction and extracted ion current profile plotting. The comparatively greater sensitivity of electrospray and low energy deposition in the ionized analyte molecules can provide high sensitivity and selectivity for the structure elucidation of pharmaceutical compounds.

3.3. In vitro analysis

Direct MD sampling of gepirone and metabolites from rat plasma without sample preparation was performed. An MS/MS precursor screen of a rat plasma sample spiked with gepirone and two known metabolites is shown in Fig. 5. The m/z 122 precursor screen is diagnostic for compounds containing the pyrimidinyl piperazinyl substructure of gepirone. This screen indicated the $(M+H)^+$ ions of gepirone (m/z 360), 1-pyrimidinyl piperazine (m/z 165), and 3-hydroxy gepirone (m/z 376). (The ion at m/z 150 is likely to originate in a cleavage of the piperazine ring and rapidly fragments further to the m/z 122 ion.) Subsequent MS/MS product ion spectral analysis of m/z 376 (Fig. 6) was consistent with the substructures associated with 3-hydroxy gepirone metabolite. The capability of direct sampling for structure-based detection and analysis of metabolites in samples without prior sample preparation (e.g. scale-up, extraction, concentration, fractionation and purification) provides a technique which supplements previous methods integrating MD and HPLC.

The carboxypeptidase Y cleavage of peptides Substance P [37] and [Lys^3]bombesin were monitored by observing the appearance and disappearance of the $(M+H)^+$ and $(M+2H)^{2+}$ ions of the peptide reactants and cleavage products as they were produced and further cleaved. The reaction profile for Substance P is shown in Fig. 7. The full scan mass spectrum for the Substance P experiment (Fig. 8) provided a molecular weight profile of Substance P reactant (M0) and the peptide products (M1–M4) in the mixture. These results are consistent with previous results for Substance P [37].

Reaction monitoring for the carboxypeptidase Y digestion of [Lys^3]bombesin (Figs. 9 and 10), is consistent with its known structure. The enzymatic reaction product profile was visualized from the appearance and disappearance of the molecular ions of the enzymatic reaction products. The system was found to be useful for monitoring C-terminal sequential cleavage of three to six amino acids of these and other peptides. The dialysate trapped within the MD membrane excludes carboxypeptidase Y, thus stopping further enzymatic reaction of the dialysate components and preserving an accurate sample from each time-point in the reaction. These results illustrate the utility for in vitro enzymatic reaction profiling for the identification of reaction product structures and short-lived intermediates.

Studies above dealing with the detection of gepirone in plasma, indicated the potential for monitoring small molecule binding to plasma components. Monitoring of ligand binding to proteins was investigate using BME and avidin. Fig. 11 shows the increase of BME $(M+H)^+$ and $(M+NH_4)^+$ ion signals as BME was sequentially added to the buffer. A stepwise decrease in BME ion signal was observed as avidin was sequentially added to the buffer. It is likely that binding of BME to avidin removed BME from the dialyzable solution and the BME–avidin complex was not allowed to pass through the dialysis membrane into the dialysate for mass spectral detection. This study indicates potential for solution phase ligand binding study.

4. Conclusions

Methods for the on-line integration of MD sampling with MS and MS/MS using thermospray and electrospray interfaces was developed. Coupling of MD sampling with thermospray and electrospray interfaces was found to provide useful levels of sensitivity, efficiency, linearity and structural identification data, consistent with current MS protocols used for pharmaceutical research and development. Short sampling times and continuous analysis were readily obtained. Applications include in vitro

pharmaceutical research experiments dealing with complex fluid matrices for drug metabolite profiling, enzymatic reaction product monitoring and protein binding profiles. Rapid analysis of samples is obtained in minutes without experimental steps for sample storage, scale-up, extraction, fractionation or individual component analysis. Dynamic in vitro reactions have been observed on-line.

Acknowledgements

The authors gratefully acknowledge Ira Rosenberg and Jerry Allison for the support and valuable resources they provided. The authors also thank Robyn Rourick and Jeff Whitney for helpful discussions on the manuscript.

References

[1] W.H. McFadden, Techniques of Combined Gas Chromatography/Mass Spectrometry: Applications in Organic Analysis, Wiley-Interscience, New York, 1973.
[2] L.R. Snyder, J.J. Kirkland, Introduction to Modern Liquid Chromatography, 2nd ed., Wiley, New York, 1979, pp. 694–706.
[3] J.V. Johnson, R.A. Yost, Anal. Chem. 57 (1985) 758A–768.
[4] K.L. Busch, G.L. Glish, S.A. McLuckey, Mass Spectrometry/Mass Spectrometry: Techniques and Applications of Tandem Mass Spectrometry, VCH, New York, 1988.
[5] F.W. McLafferty, Tandem Mass Spectrometry, Wiley, New York, 1983.
[6] M.S. Lee, R.A. Yost, Biomed. Mass Spectrom. 15 (1988) 193–205.
[7] M.S. Lee, R.A. Yost, R.J. Perchalski, in: D.M. Bailey (Ed.), Annual Reports in Medicinal Chemistry, 21, Academic Press, New York, 1986, pp. 313–330.
[8] E.H. Kerns, R.A. Rourick, K.J. Volk, M.S. Lee, J. Chromatogr. B 698 (1997) 133–145.
[9] M.S. Lee, E.H. Kerns, M.E. Hail, J. Liu, K.E. Volk, LC/GC 15 (1997) 542–558.
[10] K.J. Volk, S.E. Klohr, R.A. Rourick, E.H. Kerns, M.S. Lee, J. Pharm. Biomed. Anal. 14 (1996) 1663–1674.
[11] R.A. Rourick, K.J. Volk, S.E. Klohr, T. Spears, E. H. Kerns, M.S. Lee, J. Pharm. Biomed. Anal. 14 (1995) 1743–1752.
[12] M.S. Lee, D.J. Hook, E.H. Kerns, K.J. Volk, I.E. Rosenberg, Biol. Mass Spectrom. 22 (1993) 84–95.
[13] E.H. Kerns, K.J. Volk, S.E. Hill, M.S. Lee, J. Nat. Prod. 57 (1994) 1391–1403.
[14] M.S. Lee, S.E. Klohr, E.H. Kerns, K.J. Volk, J.E. Leet, D.R. Schroeder, I.E. Rosenberg, J. Mass Spectrom. 31 (1996) 1253–1260.
[15] J.R.M. Delgado, et al., Arch. Int. Pharmacodyn. Ther. 9 (1971) 231–235.
[16] U. Ungerstedt, C. Pycock, Bull. Schwiez. Akad. Wiss. 30 (1974) 44–51.
[17] U. Ungerstedt, in: C.A. Marsden (Ed.), Measurement of Neurotransmitter Release In Vivo, Wiley, New York, 1984, pp. 81–90.
[18] I. Jacobson, M. Sandberg, A. Hamberger, J. Neurosci. Methods 15 (1985) 263–275.
[19] W.H. Church, J.B. Justice Jr., Anal. Chem. 59 (1987) 712–721.
[20] B.H.C. Westerink, Trends Anal. Chem. 11 (1992) 176–192.
[21] S.D. Menacherry, J.B. Justice, Anal. Chem. 62 (1990) 597–606.
[22] S.A. Wages, Anal. Chem. 58 (1986) 1649–1653.
[23] D.O. Scott, L.R. Sorensen, C.E. Lunte, J. Chromatogr. 506 (1990) 461–472.
[24] C.E. Lunte, D.O. Scott, P.T. Kissinger, Anal. Chem. 63 (1991) 773A–780.
[25] B.H.C. Westerink, G. Damsma, H. Rollema, J.B. De Vries, A.H. Horn, Life Sci. 41 (1987) 1763–1777.
[26] T. Azekawa, A. Sano, K. Aoi, H. Sei, Y. Morita, J. Chromatogr. 530 (1990) 47–66.
[27] R.M. Caprioli, S. Lin, Proc. Natl. Acad. Sci. USA 87 (1990) 240–255.
[28] S. Lin, R.M. Caprioli, in: Proc. 36th ASMS Conference on Mass Spectrometry and Allied Topics, 1988, p. 1000.
[29] S. Lin, S. Chang, R.M. Caprioli, in: Proc. 38th ASMS Conference on Mass Spectrometry and Allied Topics, 1990, p. 1012.
[30] S. Lin, J.M. Slopis, P. Andrén-Johansson, S. Chang, I.J. Butler, R.M. Caprioli, in: Proc. 39th ASMS Conference on Mass Spectrometry and Allied Topics, 1991, p. 583.
[31] P.E. Johansson, R.M. Caprioli, in: Proc. 38th ASMS Conference on Mass Spectrometry and Allied Topics, 1990, p. 1327.
[32] L.J. Deterding, K. Dix, L.T. Burka, K.B. Tomer, Anal. Chem. 64 (1992) 2636–2644.
[33] L.J. Deterding, K. Dix, L.T. Burka, K.B. Tomer, in: Proc. 41st ASMS Conference on Mass Spectrometry and Allied Topics, 1993, p. 50a.
[34] L.J. Deterding and K. Tomer, in: Proc. 40th ASMS Conference on Mass Spectrometry and Allied Topics, 1992, p. 547.
[35] P.E. Andrén, R.M. Capriolli, J. Mass Spectrom. 30 (1995) 817–834.
[36] P. Michelsen, G. Pettersson, J. Rapid Commun. Mass Spectrom. 8 (1994) 517–531.
[37] R.M. Caprioli, T. Fan, Anal. Biochem. 154 (1986) 596–605.