View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Identification of In Vitro Metabolites of Indinavir by "Intelligent Automated LC-MS/MS" (INTAMS) Utilizing Triple Quadrupole Tandem Mass Spectrometry

Xiao Yu, Donghui Cui, and Margaret R. Davis

Department of Drug Metabolism, Merck Research Laboratories, West Point, Pennsylvania, USA

In an effort to improve the efficiency of the TSQ 7000 LC-MS/MS system for identification of drug metabolites in biological matrices in support of drug discovery programs, a combination of instrument control language procedures for the Finnigan MAT TSQ 7000 mass spectrometer, referred to as INTAMS, were composed. INTAMS was designed to conduct unattended, automatic liquid chromatography/mass spectrometry (LC-MS) and LC-MS/MS analyses of drugs and metabolites in commonly encountered in vitro biological matrices. A novel peak detection algorithm was developed to automatically detect and record the pseudomolecular ions and retention times of chromatographic components, even if not fully resolved. This algorithm was used in combination with an automated technique for predicting the molecular weights of metabolites based on incremental changes of the molecular weight of the parent drug resulting from well-known biotransformation processes. When applied to a sample of an incubation mixture of the HIV protease inhibitor Indinavir with a rat liver S9 preparation, the results obtained by the automatic metabolite detection procedures for LC-MS and LC-MS/MS analyses in real time were the same as those which were determined manually, by a knowledgeable operator. (J Am Soc Mass Spectrom 1999, 10, 175–183) © 1999 American Society for Mass Spectrometry

In response to the increasing demands within the pharmaceutical industry to develop therapeutic agents more rapidly, a significant change has taken place in the process by which novel lead drug candidates are synthesized. The development of automated synthetic methods has resulted in a large increase in the number of drug candidates being evaluated in the discovery phase [1, 2]. It follows that the accelerated synthesis of new drug candidates has led to increased demands upon analytical methodologies used in the evaluation of those drugs. To keep pace with these demands, analytical productivity also must increase, whether it be via the acquisition of additional instrumentation and operator personnel and/or increased efficiency of the use of existing equipment and manpower.

In the quest to design drugs of high potency, selectivity, safety, and appropriate duration of action, the chemist must take into consideration drug disposition characteristics, given that these properties will determine how much of a drug reaches its site of action and how long it will remain there. Thus pharmacokinetic studies in the early stages of drug discovery make a key contribution to the process of identifying new lead compounds by providing information on oral bioavailability and drug disposition [3]. To keep pace with the increasing number of new compounds requiring pharmacokinetic evaluation, technologies for automated high throughput bioanalyses are being developed aggressively [4–7].

When the pharmacokinetic properties of a drug are found to be unsuitable, the cause often is an underlying metabolic instability. Providing biotransformation data early in the drug discovery process can assist the chemist in designing new compounds with maximal metabolic stability and also minimal toxicity. Our laboratory is involved in studies of the biotransformation of drug candidates, utilizing liquid chromatography in conjunction with tandem mass spectrometry [liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS)] as the primary analytical tool for the identification of drug metabolites in biological matrices. In the realm of drug discovery, our efforts are dedicated to metabolite identification for the fulfillment of three objectives: (1) to characterize metabolic "soft spots" in order to provide the chemist with information that will be useful in the design of more metabolically stable compounds, (2) to identify drug metabolites in order that they may be synthesized and then tested for pharmacological activity, and (3) to detect products of metabolic activation, through which reactive, poten-

Address reprint requests to Dr. Margaret R. Davis, Cerep, Inc., 15318 NE 95th Street, Redmond, WA 98052.

© 1999 American Society for Mass Spectrometry. Published by Elsevier Science Inc.
1044-0305/99/$20.00
PII S1044-0305(98)00132-9

Received February 24, 1998
Revised September 3, 1998
Accepted September 29, 1998

tially toxic metabolites are generated, and whose formation can be suppressed or eliminated by appropriate structural modification.

It was first demonstrated over a decade ago that LC-MS/MS could be utilized for the structural characterization of metabolites in biological matrices [8]. Since that time, the process of introducing samples directly into the mass spectrometer via on-line high-performance liquid chromotography (HPLC) separation (LC-MS) has proven to be considerably less time consuming and less labor intensive than off-line chromatographic isolation of metabolites prior to mass spectral analysis. In conjunction with on-line sample introduction, MS/MS capabilities have made possible the acquisition of structurally informative data from pseudomolecular ions of analytes of interest, even when they are not chromatographically resolved.

The latest generation of Finnigan MATs (San Jose, CA) triple quadrupole instruments, the TSQ 7000, features a powerful data system (ICIS) which provides a text editing utility through which operators can construct instrument control language (ICL) procedures of their own design. This utility makes accessible to the instrument operator, the software used in the control of data acquisition. This accessibility provides users with a broad variety of options for developing and customizing instrument control procedures for their own specific needs.

In 1995, Finnigan MAT reported advances made in the automation of acquisition of LC-MS/MS data by using the TSQ 7000. Three commercial ICL procedures, namely "retention time dependent," "data dependent," and "signal dependent" automated LC-MS/MS were described and made available for use (and potential modification) by the customer base [9]. Retention time dependent automated LC-MS/MS is a very useful technique for the acquisition of product ion spectra if the molecular masses and chromatographic retention characteristics of the analytes of interest are known. Obviously, a prior analysis of the sample by full scan LC-MS is required before retention time dependent LC-MS/MS can be put to good use.

Data dependent automated LC-MS/MS is a powerful technique which makes possible the acquisition of product ion spectra of analytes of known molecular weight, even if there is no available retention behavior information. Unfortunately, without prior analysis of the sample by LC-MS to determine the molecular masses of the analytes of interest, the pseudomolecular ions which the automatic LC-MS/MS procedure will "see" are limited to those which the analyst can predict. Data dependent LC-MS/MS requires full scan LC-MS analysis to be carried out during a chromatographic analysis until a predetermined ion of interest elutes, at which time the procedure switches the TSQ 7000 into product ion scanning MS/MS mode. After a predetermined number of MS/MS scans are performed, the instrument switches back to full scan MS mode, until another, or the same analyte of interest is detected. Signal dependent automatic LC-MS/MS calls for the switching from full scan LC-MS analysis to product ion scanning MS/MS mode each time the ion current signal from any chromatographic component exceeds a preset threshold value. Theoretically, this type of procedure can allow for the complete MS/MS analysis of any predictable or non-predictable metabolites in a sample in a single chromatographic run. Unfortunately, because of the large difference in ion currents generated by full scan and product ion scanning analyses, chromatograms generated by data and signal dependent automatic LC-MS/MS procedures appear disjointed and do not actually reveal discrete peaks. We have determined that, for our purposes, both a full scan LC-MS chromatographic analysis and at least one product ion scanning LC-MS/MS analysis are required for the characterization of any one sample. A full scan LC-MS analysis of each sample for metabolite identification is desirable in that such analyses generate chromatograms which are valuable visual aids for imagining a compound of interest's metabolic profile. Because of the differing ionization potentials between analytes, such ion current chromatograms do not give a reliable quantitative description of the metabolites as would a HPLC radiochromatogram. However, they can give the viewer information regarding the number of metabolites present in the matrix analyzed and whether a metabolite of interest might be present as a major or minor component, based very roughly on peak area.

In 1995, Cole et al. [10] developed an automated LC-MS/MS procedure for metabolite identification with the TSQ 7000, known as rapid automated biotransformation identification (RABID). RABID represented a breakthrough in the development of automated LC-MS/MS methodology for the rapid identification of drug metabolites. In this procedure, samples of drug metabolites generated in vivo or in vitro are subjected initially to full scan LC-MS analysis. During that analysis, RABID detects any component whose pseudomolecular ion's mass-to-charge ratio differs from that of the parent drug by a mass increment which might arise from a common biotransformation process, e.g. the addition of 16 mass units resulting from oxidation of the parent drug. RABID records the pseudomolecular ions of these predictable metabolites and their retention times from the initial full scan LC-MS analysis and utilizes that information in a second analysis which constitutes a retention time dependent automated LC-MS/MS procedure. RABID can obtain LC-MS and LC-MS/MS data on many metabolites of a drug in a complex mixture in two chromatographic analyses, with nothing known in advance about the parent drug other than its molecular weight. Automated procedures modeled after RABID have been used successfully in our laboratory but have been found lacking in their ability to detect and obtain data from metabolites such as products of *O*- and *N*-dealkylation (often major routes of biotransformation) if the structure of the

parent compound has not been studied in advance of the analyses.

Contained herein is a description of the first step we have taken to accelerate and automate metabolite identification by LC-MS/MS in our laboratory while maintaining the highest standards of quality and completeness. By building on the advances made in automated LC-MS/MS technology by Finnigan MAT and Cole *et al*. and by developing a novel, real-time peak detection algorithm, we have sought to increase the efficiency of operation of our existing TSQ 7000 LC-MS/MS systems and minimize the involvement of operators. Although increased efficiency is desirable for all aspects of our work, the procedures described here are ideally suited for the analysis of samples generated from in vitro systems and thus are readily applicable to the support of drug discovery.

Traditionally, discovery support analyses in our laboratory were carried out using the sequence of procedures listed in the following scheme, applied to samples generated by the incubation of drug candidates in appropriate in vitro systems, such as liver microsomal or S9 preparations.

1. Perform full-scan LC-MS analyses of incubation samples.
*2. Manually process chromatographic data generated from full-scan analyses. Record the retention times of chromatographic peaks observed in reconstructed total ion current chromatograms and the corresponding mass-to-charge ratios of precursor ions which give rise to those peaks.
*3. Construct an ICL procedure to generate product ion spectra of the pseudomolecular ions of the drug and its metabolites.
4. Perform the required product ion scanning LC-MS/MS analysis or analyses. (In many cases, product ion spectra can be obtained for all metabolites in a single analysis.)
*5. Manually process product ion spectral data.
6. Interpret product ion spectra to characterize the structures of metabolites.

A generic ICL procedure is used for the performance of all full scan analyses, so the initiation of Step 1 requires only the click of a mouse button. The analyses, obviously, can be carried out unattended. Once the full scan analyses are complete, however, the chromatographic data must be processed and the operator must determine which precursor ions correspond to chromatographic peaks observed in reconstructed total ion current chromatograms (Step 2). Once drug metabolite peaks are identified, the mass-to-charge ratios of their pseudomolecular ions and retention times are recorded manually. The operator then composes a retention-time dependent product ion scanning ICL procedure to obtain product ion spectra for as many metabolites as possible in one analysis (Step 3). Once product ion scanning ICL procedures have been generated, the analyses can be carried out unattended (Step 4). After the data have been collected, they are processed manually (Step 5). Step 6 involves the interpretation of product ion spectra for the characterization of metabolites and, at present, must be done manually by an analyst with significant expertise in spectral interpretation and a sound knowledge of biotransformation processes.

Steps 2, 3, 5, and 6 (marked with an asterisk) traditionally require operator involvement and can be labor intensive and time consuming. Obviously, if there is no operator present, as there tends not to be during the overnight hours, the analysis sequence cannot proceed from Step 2 to Step 3 and beyond. It was the goal of the work described here to automate Steps 2 and 3 to reduce the amount of labor involved in their execution and to permit the analysis sequence to proceed, unattended, from Step 1 through Step 4. Achievement of the goal will result in reduction of the manpower requirement and the extent of overnight delays involved in metabolite identification.

To demonstrate the capabilities of the automated procedures described here, the Merck HIV protease inhibitor, Indinavir. (*N*-[2(*R*)-hydroxy-1(*S*)-indanyl]-5-[2(*S*)-tertiary-butyl-aminocarbonyl-4-(3-pyridylmethyl)piperazino]4(*S*)-hydroxy-2(*R*)-phenylmethylpentanamide, Crixivan, Figure 1) was chosen as a model compound to generate metabolites for detection. The metabolism of Indinavir in several species has been reported [11, 12].

Experimental

Biological Methods

Indinavir was incubated with rat liver S9 fractions at a substrate concentration or 20 μM and a protein concentration of 5 mg mL^{-1} in 100 mM potassium phosphate buffer at pH 7.4. Reactions were initiated by the addition of nicotinamide adenine dinucleotide phosphate reduced form (NADPH) to give a final cofactor concentration of 1 mM. Each incubation mixture (1 mL total volume) was shaken in a water bath kept at 37°C for 1 h. Reactions were terminated by the addition of two volumes of ice-cold acetonitrile. The samples were then vortex mixed and centrifuged. The supernatants were dried and the residues reconstituted in a 1 mL volume of 0.1% formic acid in water and acetonitrile (95:5, v/v). Aliquots (15 μL) then were injected onto HPLC.

HPLC

HPLC was carried out on a Hewlett-Packard HP1050 gradient HPLC system consisting of a solvent delivery system and an autosampler. Separation was carried out on an Ultrasphere (Beckman, Fullerton, CA) C_{18} column

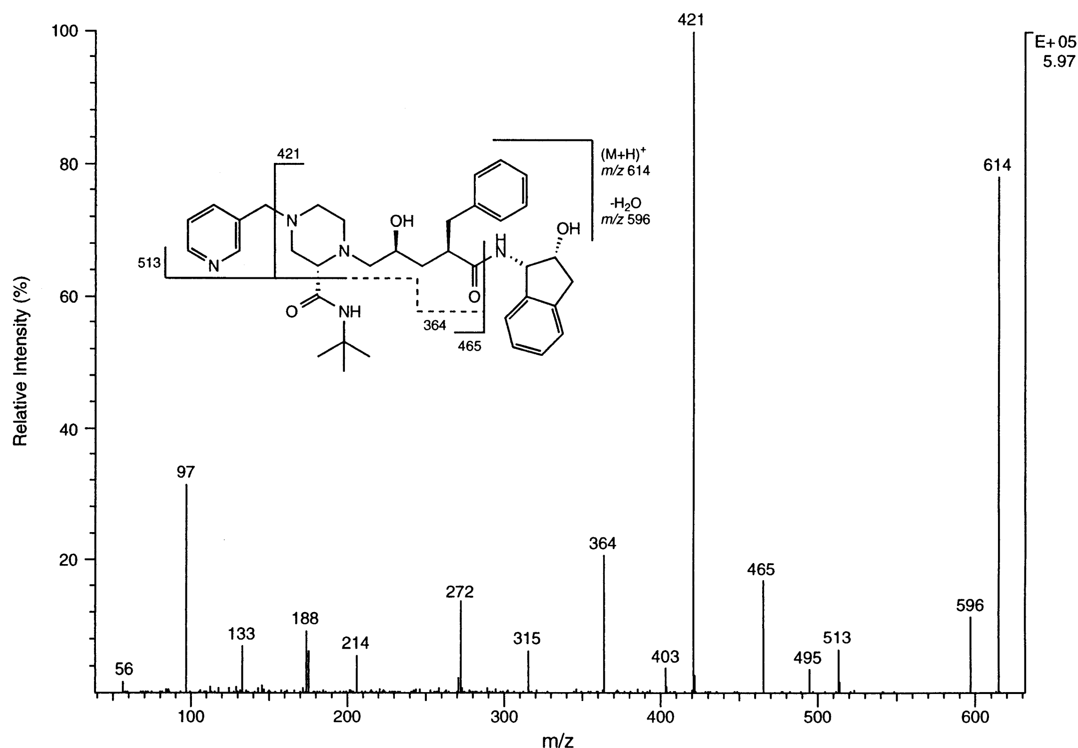

Figure 1. Product ion spectrum generated upon CID of the $(M + H)^+$ ion (m/z 614) of Indinavir (Crixivan). Proposed origins of product ions are also shown.

(2 mm × 15 cm, 5 μ) using a mobile phase consisting of 0.1% formic acid in water (solvent "A") and 0.1% formic acid in acetonitrile (solvent "B") and a constant flow rate of 0.2 mL min^{-1}. The gradient used was as follows: Isocratic conditions of 5% B were maintained for 2 min followed by a linear increase to 20% B in 3 min. This was followed by a second linear increase to 50% B in 35 min. The column then was washed with 80% B for 3 min and equilibrated at 5% B for 15 min prior to each injection.

Mass Spectrometry

The HPLC system described was interfaced to a Finnigan TSQ 7000 tandem mass spectrometer. Both the mass spectrometer and the HPLC were controlled from a DEC 3000 Alpha station operating with ICIS Version 8.2. Mass spectral analyses were carried out using electrospray ionization (ESI) in the positive ion mode, with the ESI ionizing voltage maintained at 5.0 kV for all analyses. The temperature of the heated capillary inlet was maintained at 230 °C and the multiplier voltage was 1700 V. For full scan analyses, the first quadrupole was scanned from m/z 180 to m/z 850 in 0.7 s. A scan range lower limit of m/z 180 was selected to preclude the observation of solvent cluster ions which might otherwise appear as base peak ions in the full scan mass spectra. Tandem mass spectrometry (MS/MS) was based on collision-induced dissociation (CID) of ions entering the rf-only octapole region where argon was used as the collision gas at a pressure of 1.7 mtorr. All product ion scans were carried out over the mass range of m/z 50 to a value 10 Da greater than the mass of the precursor ion, with a scan time of 0.5 s. The optimal collision offset (E_{CID}) for Indinavir, defined as the collision energy required to reduce the intensity of the precursor ion ($[M + H]^+ = m/z$ 614) to ~30% of that of the base peak in the product ion spectrum (m/z 421, Figure 1), was determined to be −30 eV.

Results and Discussion

As stated previously, one of the advantages that the Finnigan TSQ 7000 offers over other commercially available MS/MS systems is the capability of writing ICL procedures that can control the mass spectrometer in the performance of user-customized tasks. The electronics of the TSQ 7000 system allow for rapid parameter switching on time scales which are compatible with those used typically in routine HPLC separations. Tables, referred to as "user lists," are available to the instrument's operator and serve as interactive data bases in which data can be stored temporarily, analyzed mathematically and subsequently used for control of the instrument's acquisition parameters. The INTAMS ("Intelligent Automated LC-MS/MS") procedure described here is composed of two programs (packages of ICL procedures written in our laboratory) and requires

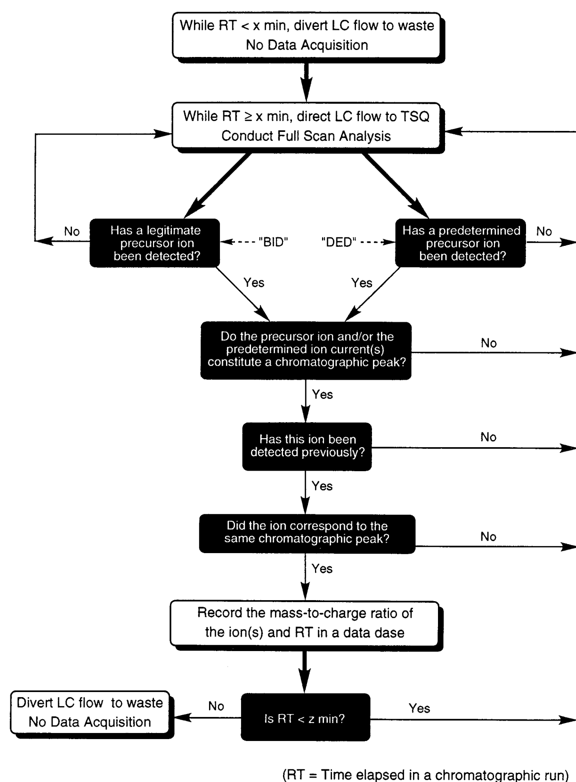

Figure 2. Logic diagram of a package of instrument control language procedures used for the automatic acquisition and real-time analysis of LC-MS data. The program performs a peak detection task which also records pseudomolecular ions and elution start times of drugs and metabolites in vitro.

two separate chromatographic analyses for each sample. The first program is executed during the full scan analysis of the first chromatographic run of a particular sample and is actually a decision-making program. A logic diagram of this procedure is shown in Figure 2. While the mass spectrometer is conducting a full scan analysis during the first chromatographic run, the program records the mass-to-charge ratio and the intensity associated with the base peak ion from each scan in a continuously updated data base. After performing an arithmetic data smoothing procedure over overlapping series of scans in real time, the program performs a peak detection task in order to determine if the ion current from the base peak of a series of scans actually constitutes a "chromatographic peak." This peak detection task involves performing an arithmetic manipulation on the smoothed ion current signals and making corrections for noise on these signals at each point in time. Intensities of individual smoothed data points are denoted I_i, which in any single case is the average intensity of the signal of the base peak ion in scan numbers i-4, i-3, i-2, i-1 and i, provided the mass-to-charge ratio of the base peak ion in each of these scans is constant. Noise is determined arithmetically at each scan number i (N_i) by averaging the intensity of the tenth most abundant peak in each full scan spectrum

Table 1. Mass-to-charge ratios of predetermined metabolites (expressed as functions of the molecular weight of the parent drug) and the common biotransformation process(es) from which they might be derived

Metabolite precursor ion m/z (addition to $[M + H]^+$)	Type of biotransformation (Phase I)
+16	Addition of oxygen
+14	Addition of oxygen & two-electron oxidation
+30	Addition of oxygen (2) & two-electron oxidation
+32	Addition of oxygen (2)
+18	Hydration
+36	Hydration (2)
−2	Two-electron oxidation
+2	Two-electron reduction
−14	Demethylation

over the preceding 20 scans. Peak detection criteria are fulfilled if the ratio of a smoothed, noise-corrected base peak ion intensity at any scan number (i + 5), and the same value determined for the corresponding scan number i, referred to as $S_{(i+5)}$, equals or exceeds a minimum value of 1.4, i.e.

$$S_{(i+5)} = \frac{(I_{(i+5)} - N_{(i+5)})}{(I_i - N_i)}$$

If $S_{(i+5)}$ is determined at any point in a LC-MS analysis to equal or exceed a value of 1.4, INTAMS records the mass-to-charge ratio of the recurring base peak ion and the retention time corresponding to scan (i + 5) in a data base. That data base is accessed during a second chromatographic analysis of the same sample. The process, used for the detection of base peak metabolite precursor ions has been given the abbreviation BID ("base peak ion detection"). BID is also capable of detecting components of mixtures which are not well-resolved chromatographically. If the intensity of the second most intense ion in any particular scan reaches 25% of that of the base peak, BID will record that ion's mass-to-charge ratio in the data base and perform the same smoothing and peak detection tasks as were carried out on the base peak ion. If the chromatographic peak criteria are met by the second most intense ion, BID records the mass-to-charge ratio of the ion and its associated elution-start time in the data base as well.

While BID is being executed, an additional process, referred to as DED (predetermined precursor ion detection), searches for any predictable metabolite precursor ions that might not appear as base peak ions. Predetermined metabolite precursor ion mass-to-charge ratio values are chosen to reflect common pathways of xenobiotic metabolism. Table 1 shows a list of formulae for the determination of mass-to-charge ratios of such pseudomolecular ions as a function of the molecular weight of the parent drug. Also shown are the metabolic processes from which such metabolites might be

derived. During the initial LC-MS analysis, signals produced from any such ions are subjected to the same peak detection analysis by DED as were base peak ions by BID. If any predetermined metabolite precursor ions are found to constitute actual chromatographic peaks, their mass-to-charge ratios and elution-start times are recorded in the same data base as were the peaks detected by BID.

In total, BID and DED produce a data base which lists the mass-to-charge ratios of the base peak ions of all components, second most intense peaks which are at least 25% as abundant as the base peak and also any predetermined metabolite precursor ions which correspond to actual chromatographic peaks, whether or not they are fully resolved chromatographically. Recorded along with these values is the run time at which each legitimate chromatographic entity began to elute. If there are any results duplicated by the two peak detection functions, the second entry is automatically ignored by the retention-time dependent product ion scanning algorithm which is carried out during the second chromatographic analysis of the sample. This is made possible by a postacquisition "clean-up" ICL procedure composed in our laboratory which simplifies the data base generated during the INTAMS full scan LC-MS analysis. This procedure converts table entries which have closely similar retention times and precursor ions of identical nominal mass to a single table entry. This procedure has proven to be key to the successful application to INTAMS.

Because there are two different precursor metabolite detection sensors functioning simultaneously during the first chromatographic analysis, the program is able to detect chromatographic peaks which are only partially separable by chromatography and many components which actually co-elute. The bottom trace in Figure 3 depicts a reconstructed total ion current chromatogram which was generated manually following the completion of a full-scan LC-MS analysis of an extract from a rat liver S9 incubation with Indinavir. The ion current traces above it were extracted manually by an operator who was searching for chromatographic peaks generated from base peak ions and from pseudomolecular ions of predicted metabolites. Chromatographic peaks which were determined by the operator to be unrelated to the parent drug, by visual comparison with the ion current trace generated from analysis of a control sample (an incubation mixture containing all cofactors but no drug), were not extracted for display. All of the peaks from the full scan LC-MS analysis which were determined by the operator to be pertinent also were detected by either BID or DED and are shown (in addition to those from some non-drug-related components) in a data base in Table 2. It is apparent that BID and DED did not "miss" any metabolites which were detectable by the operator.

A second package of ICL procedures was written to conduct automatic LC-MS/MS analysis of an incubation extract during a second chromatographic run. This second program automatically accesses the data base which was generated by BID and DED during the previous full-scan LC-MS analysis and performs product ion scanning of pseudomolecular ions of the parent drug and its metabolites. From the data base, the second program selects the pseudomolecular ions from which product ion spectra are to be generated during the run-time interval in which each component is expected to elute. A logic diagram of this automated LC-MS/MS procedure is shown in Figure 4.

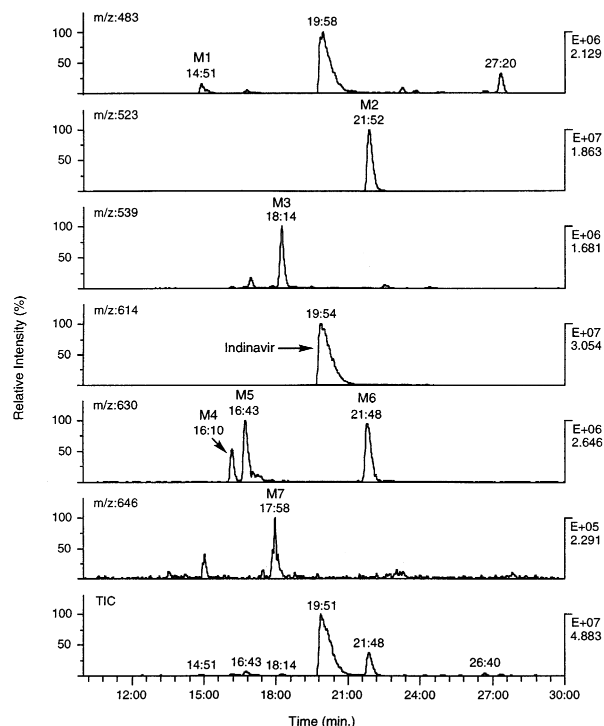

Figure 3. Reconstructed ion current chromatograms generated from full scan LC-MS analysis of an incubation of Indinavir with rat liver S9. The bottom trace represents the total ion current. The other single-ion current traces were manually extracted by an operator who selected mass-to-charge ratios corresponding to base peak ions and pseudomolecular ions of predicted metabolites.

INTAMS allows for the mass range scanned by the third quadrupole to be automatically adjusted for each individual precursor ion. In order to accommodate the possible shifts in retention times between runs, an adequate acquisition window width is automatically set for each ion. If a reference value is provided before the analysis for the optimal collision energy (E_{cid}) for the parent drug, rotating collision offset values of $E_{cid} - 15$ V, E_{cid} and $E_{cid} + 15$ V are used to provide the maximum number of interpretable product ions from each eluting component. Product ion scans of overlapping series of metabolite precursor ions in the data base are carried out until the retention time is within 0.3 min of

Table 2. A data base generated by the intelligent peak detection sensors, BID and DED, during a full scan LC-MS analysis of an incubation of Indinavir with rat liver S9; the data were accessed automatically by a product ion scanning program in a second chromatographic analysis of the same sample

Precursor ion (m/z)	Elution start time (min)
377.098[a]	12.441
475.913[a]	12.720
319.043[a]	12.866
469.395[a]	13.107
564.475[a]	13.493
483.332[a]	14.689
630.200[a,b]	15.952
391.257[a]	16.352
630.200[a,b]	16.552
646.200[b]	17.807
539.372[a]	18.099
614.472[a]	19.255
630.200[b]	21.587
523.343[a]	21.681
505.368[a]	24.142

[a]Detected by BID.
[b]Detected by DED.

the elution start time of the next metabolite precursor ion in the data base (Figure 4). In this manner, possible shifts in retention time between runs are taken into consideration.

Figure 5 shows proposed structures, product ion spectral data and spectral interpretations corresponding to rat liver S9 metabolites of Indinavir, M1–M6, respectively. In addition to three products of monohydroxylation of the parent drug, two *N*-dealkylation products and a product of both *N*-dealkylation and mono-hydroxylation were observed. A seventh metabolite (dihydroxylated product, M7) was detected but the information derived from the corresponding product ion spectrum was insufficient for structural characterization. Product ion spectra of other components were generated by INTAMS, but interpretation of the spectra led to the conclusion that these components were not drug related. The findings of the metabolite identification exercise described here correlate well with the findings from other laboratories [11, 12].

INTAMS has been demonstrated to eliminate the requirement for operator involvement during the process of collecting LC-MS and LC-MS/MS data in mixtures generated from in vitro incubations of drugs, without compromising the quality of the results. If a time of 45 min is assumed for each chromatographic analysis, without the use of INTAMS, an operator can extract metabolite precursor ions from LC-MS data and write retention time dependent ICL procedures for approximately ten samples per work day. The LC-MS/MS analyses of those same samples can be carried out overnight to complete the full analyses of 10 samples in about 8 additional hours. This amounts to the full analysis of 10 samples in a 24 hour period. With the availability of INTAMS and an assumed run time of 45 min, at least 15 samples can be fully analyzed in a 24 hour period. In addition, no operator involvement is required in the process after initiating the batch of analyses. This gives the operator the freedom to perform other tasks while the analyses are being carried out, such as interpretation of data acquired the previous day. The use of INTAMS for sample analysis allows the operator to spend a much larger fraction of working hours devoted to spectral interpretation, which currently is the rate-limiting process in metabolite identification. Devoting a larger fraction of the available manpower to "the bottleneck" has had a profound impact on the overall productivity of our laboratory.

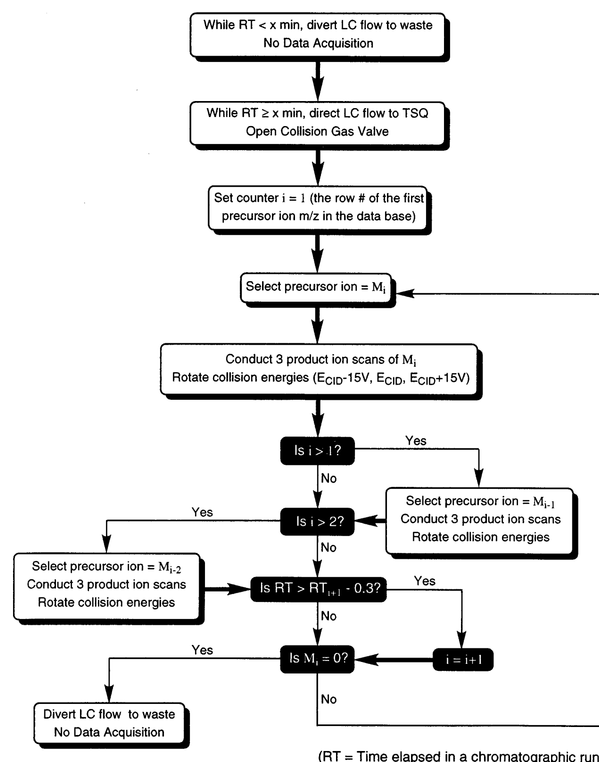

Figure 4. Logic diagram of a program used for automated product ion scanning of pseudomolecular ions of drugs and metabolites in biological media. From a data base generated during a previous full scan LC-MS analysis of the same sample, this program selects precursor ions for MS/MS analysis during the run-time interval in which each peak is expected to elute.

INTAMS, as it has been described here, has been used in our laboratory since March 1997, and has doubled our laboratory's production of data with respect to the support of drug discovery programs. The preliminary version of INTAMS described here, which involves full scan LC-MS analysis as the first step, is likely to be applicable only to samples where few endogenous substances are found to co-elute with

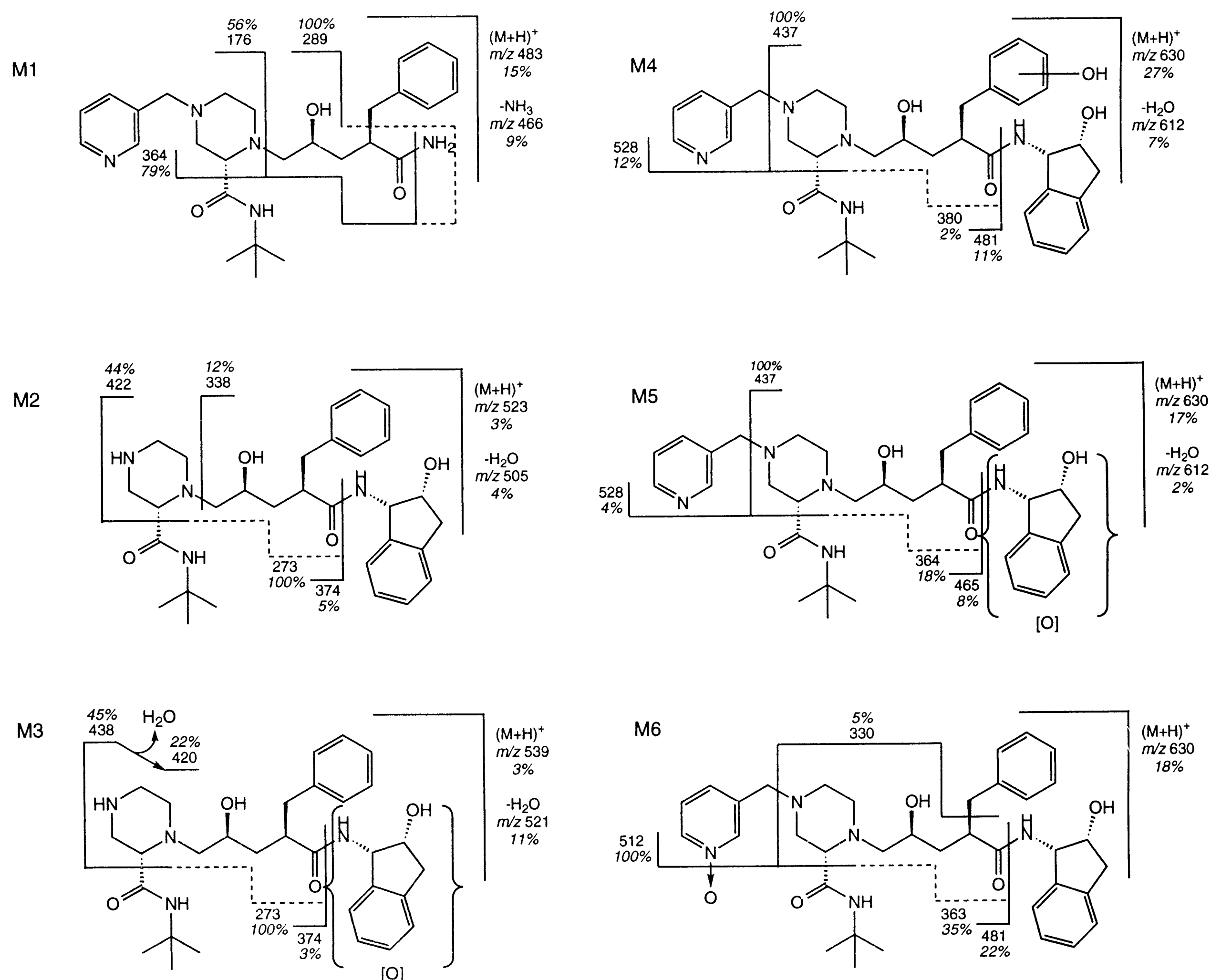

Figure 5. Proposed structures of six rat liver S9 metabolites of Indinavir (M1–M6). Proposed origins of major product ions (relative intensities in italics) generated upon CID of the respective $(M + H)^+$ ion are also shown.

drug-related materials and the drug-related components are present in concentrations which are high in comparison to the concentration of endogenous substances. To be useful in the identification of drug metabolites in samples generated from studies carried out in vivo, such as urine or bile, scan modes more specific than full scan analysis may need to be used for the initial, metabolite detection phase of INTAMS. INTAMS is currently under further development in our laboratory for potential use in the identification of in vivo drug metabolites. Promising results have been obtained, thus far, using methods of scanning for precursor ions and and/or neutral losses characteristic of the MS/MS behavior of the parent drug (and some predictable metabolites) as means of detecting drug-related materials in samples from in vivo studies.

Acknowledgments

The authors gratefully acknowledge support of this work by Dr. Thomas A. Baillie and the late Dr. John D. Gilbert of the Department of Drug Metabolism, Merck Research Laboratories.

References

1. Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P.; Gordon, E. M. *J. Med. Chem.* **1994,** *37*, 1233–1251.
2. Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P.; Gallop, M. A. *J. Med. Chem.* **1994,** *37*, 1385–1401.
3. Humphrey, M. J. *Drug Metab. Rev.* **1996,** *28*, 473–489.
4. Halm, K. A.; Adkison, K. K.; Berman, J.; Shaffer, J. E.; Tong, Q. W.; Lee, F. W.; Unger, S. E. Proceedings of the 44th ASMS Annual Conference on Mass Spectrometry and Allied Topics; Portland, OR, May 1996; p 392.
5. Olah, T. V.; McLoughlin, D. A.; Gilbert, J. D. *Rapid Commun. Mass Spectrom.* **1997,** *11*, 17–23.

6. McLoughlin, D. A.; Olah, T. V.; Gilbert, J. D. *J. Pharm. Biomed. Anal.* **1997,** *15*, 1893–1901.
7. Berman, J.; Halm, K. A.; Adkison, K. K.; Shaffer, J. E. *J. Med. Chem.* **1997,** *40*, 827–829.
8. Rudewicz, P.; Straub, K. M. *Anal. Chem.* **1986,** *58*, 2928–22934.
9. Mylchreest, I.; Wheeler, K.; Campbell, C. Finnigan MAT TSQ® Application Report **1995,** #240.
10. Cole, M. J.; Gauthier, J. W.; Luther, E. W.; Fouda, H. G. *Proceedings of the 43rd ASMS Annual Conference on Mass Spectrometry and Allied Topics*; Atlanta, GA, May 1995; p 569.
11. Lin, J. H.; Chiba, M.; Balani, S. K.; Chen, I.-W.; Kwei, G. Y.-S.; Vastag, K. J.; Nishime, J. A. *Drug Metab. Dispos.* **1996,** *24*, 1111–1120.
12. Balani, S. K.; Woolf, E. J.; Hoagland, V. L.; Sturgill, M. G.; Deutsch, P. J.; Yeh, K. C.; Lin, J. H. *Drug. Metab. Dispos.* **1996,** *24*, 1389–1394.