A new fully automated mass spectrometer for confirmative analyses in drug development

Karl-Heinz Maurer[1], Harald Gerken[1], Martin Maurer[1], Heinz Musche[2]

[1] AMD Intectra GmbH, Königsberger Str. 1, D-27243 Harpstedt, Germany
Phone: +49-4244-1062; Fax: +49-4244-8646;
e-mail: amd.intectra@t-online.de; web site: http://www.amd-intectra.de

[2] Bayer AG, PH-R-Structural Research, D-42096 Wuppertal, Germany
Phone: +49-202-36-4207; Fax: +49-202-36-8552

ABSTRACT

An AMD M-40 DF magnetic sector mass spectrometer of benchtop design is used in combination with a Zymate™ robot system to provide high throughput MS analyses in Direct Chemical Ionization (DCI)-mode.

The major analytical target of this system is automatic confirmation of molecular weights in combinatorial synthesis under high sample throughput conditions. The DCI technique provides unequivocal molecular weight confirmations with very high statistical probabilities and proves to be a very efficient and complementary method to ESI techniques. For a number of substance classes the DCI technique is the method of choice.

A unique API/EI Ion Source for direct ESI analyses and LC/MS interfacing is available for the AMD M-40 series of mass spectrometers, too.

The system functions are described and results are presented.

INTRODUCTION

AMD Intectra GmbH is a manufacturer of high performance magnetic sector mass spectrometers. The recently introduced benchtop designed series of AMD M-40 systems is well suited for an automatic operation in a wide range of applications (GC/MS, LC/MS, DCI-MS, DEI-MS, FD/FI-MS, etc). Besides other mass spectrometric ionization techniques the Pharmaceutical Research department at Bayer AG has been using the Direct Chemical Ionization technique for many years to confirm the expected molecular weight of a synthesized drug. Due to new combinatorial chemistry techniques the number of samples which have to be analysed by (DCI) mass spectrometry was increased. The utilization of automated analyses is neccessary in order to cope with today´s requirements in which time and cost efficiency are important aspects.

AMD M-40 SERIES OF MASS SPECTROMETERS

Figure 1: AMD M-40 Mass Spectrometer

The AMD M-40 series of magnetic sector mass spectrometers in benchtop design combine the advantages of sector instruments with the ease of operation of quadrupole systems.

The basic version is a single focusing instrument with low resolution capabilities. This system can be upgraded with an electric sector to form a double focusing configuration with better resolution and MS/MS capabilities. The more powerful AMD M-40 S offers high resolution capabilities with accurate mass determination as well as high specificity and low limits of detection, especially in combination with chromatographic techniques. The system described in this paper is based on the double focusing version AMD M-40 DF (Figure 1). Table 1 contains a summary of the key specifications of the different versions.

DIRECT CHEMICAL IONIZATION (DCI)

Direct **C**hemical **I**onization describes a soft ionization technique which is deduced from Chemical Ionization (CI) methodology in which sample molecules are in the gas phase when they are introduced into the CI-plasma.

In DCI-mode a heatable wire (up to 1000°C), coated with the sample, is introduced directly into the ion source in which a CI-plasma is formed at a pressure of 0.2-1 torr of a reactant gas. The chemical reaction between reactant ions and sample molecules desorbed from the wire effects the production of quasi-molecular ions (adduct ions) without significant fragmentation.

The quasi-molecular ions are used for confirmation of the molecular weight of the analysed sample. The DCI technique is complementary or substitutional to other

soft ionization methods as Electrospray (ESI), Field Desorption (FD) and Fast Atom Bombardment (FAB).

Table 1: AMD M-40 Series of Mass Spectrometers

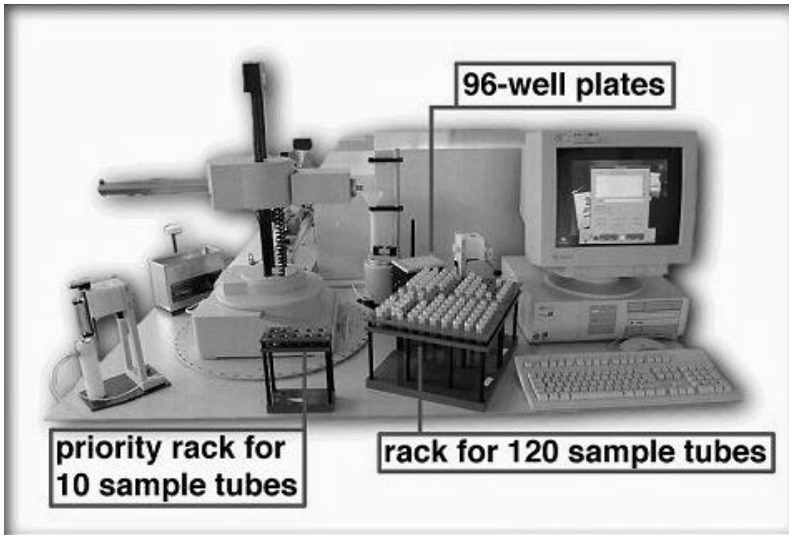
Description	AMD M-40	AMD M-40 DF	AMD M-40 S
Analyzer	single focusing magnetic sector mass spectrometer	double focusing magnetic sector mass spectrometer in BE configuration	double focusing magnetic sector mass spectrometer in BE configuration
Key Specifications			
Mass Range	2,300 at 6 kV accelerating voltage	2,300 at 6 kV accelerating voltage	2,300 at 6 kV accelerating voltage
Resolution	> 2 000 (20% valley definition)	> 4 000 (10% valley definition)	> 15 000 (10% valley def.)
Sensitivity (EI)	> 2 x 10^{-7} C/µg (R = 500, methyl stearate)	> 2 x 10^{-7} C/µg (R = 1 000, methyl stearate)	> 3 x 10^{-7} C/µg (R = 1 000, methyl stearate)
Accuracy of Mass Determination	accurate mass measurements not available	computer aided accurate mass determination (V/E-Scan): 10 ppm at R = 2 000	computer aided accurate mass determination (V/E-Scan): 4 ppm at R = 5 000
Ionization Techniques	all ionization techniques are available for all instruments: EI/CI, ESI/APCI, Liquid SIMS (FAB Pendant), DCI, FI/FD		
Sample Introduction	all sample introduction systems and chromatography techniques are available for all instruments: DIP, GC/MS, LC/MS, etc.		
Special System Features	Unique Combination API/EI-Ion Source for alternating operation in all modes. EI/CI ion source and ESI/APCI interface are mounted in an axial configuration and connected by ion transfer optics.		
Options	MS/MS techniques Linked Scans (B/E, B^2/E, Constant Neutral Loss) and MIKES, multi sector MS/MS configurations		

AUTOMATED SAMPLE PREPARATION

The Zymate™ system (Figure 2) prepares the samples which are delivered either in screwed vials or in 96-well microplates automatically for mass spectrometric analyses. In this paper we describe the preparation of solid samples delivered in screwed vials.

An Excel based table contains the information concerning the sample (e.g. position at the rack, to be dissolved (yes/no), expected mass of the molecular ion (M), sample name, file documentation number, etc) which has been typed by the user or via bar-code reader. A special program transfers the required information from the Zymate™ system to the data system of the mass spectrometer. In this way it is ensured that the data of the prepared sample are assigned correctly to the data of the analyzed sample.

Figure 2: Picture of the Zymate™ System



The Zymate system includes:

Pentium PC as control processor for data input and program start
Zymate XP robot
Gripper hand for screwed vials
Syringe hand with washing station
Solvent dosing station
Ultra sonic bath for dissolving assistance
Sample rack for 120 vials with screw cap
Sample rack for 10 priority vials with screw cap
Capper to unscrew and screw the vials
96-well microplate station

SAMPLE PREPARATION CYCLE

The robot takes the gripper hand and moves a sample from the sample rack to the capper station to unscrew the vial (Figure 3). If the sample is not yet dissolved, the vial is being moved to the dosing station to add solvent and then the ultra sonic bath assists the dissolving (Figure 4). The vial is now placed at the capper station to allow the robot to change from the gripper hand to the syringe hand (Figure 5). After having cleaned the syringe, the robot fills the syringe with the dissolved sample and moves to the automated sample introduction device of the mass spectrometer to transfer the sample to the DCI wire (Figure 6). Before transferring the sample, the robot gets a confirmation that the mass spectrometer is ready. After the sample was transferred, the syringe will be cleaned and the robot changes to the gripper hand to screw the vial and to place it at the original position at the rack. The robot checks now the priority rack and in case a vial was placed there the robot will go on with this sample according to the above described

procedure, otherwise the next sample from the regular rack will be prepared for the mass spectrometric analysis.

Figure 3: Pictures from the sample rack and the capper station

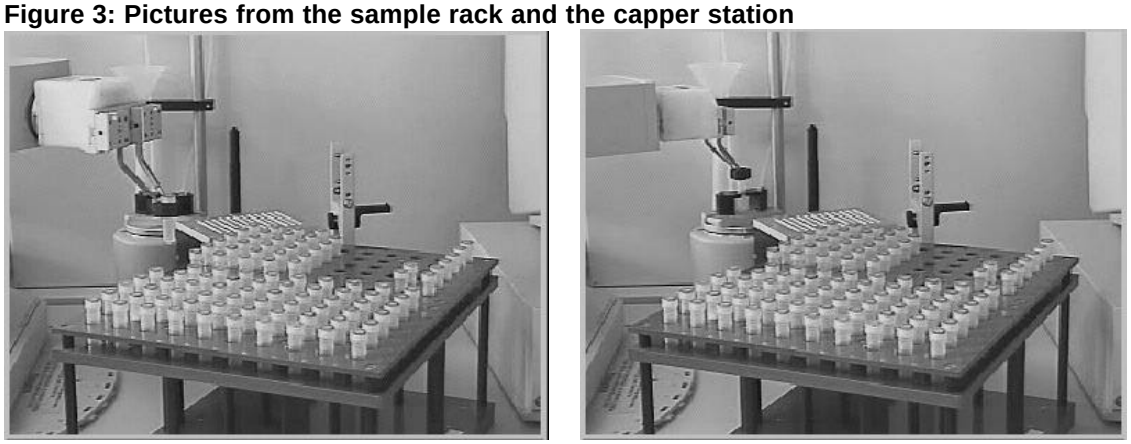


Figure 4: Pictures from the dosing station and the ultra sonic bath

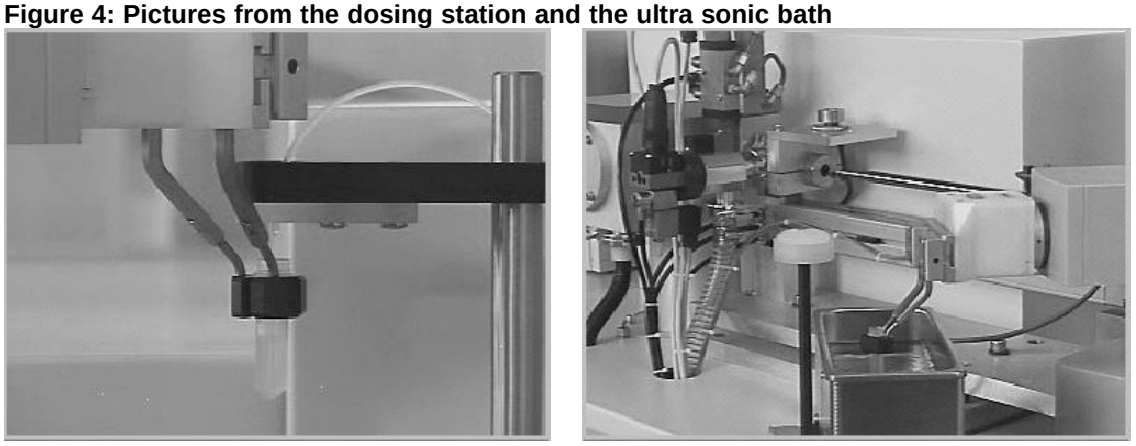


Figure 5: Pictures of drying the outside of the vial and following hand exchange

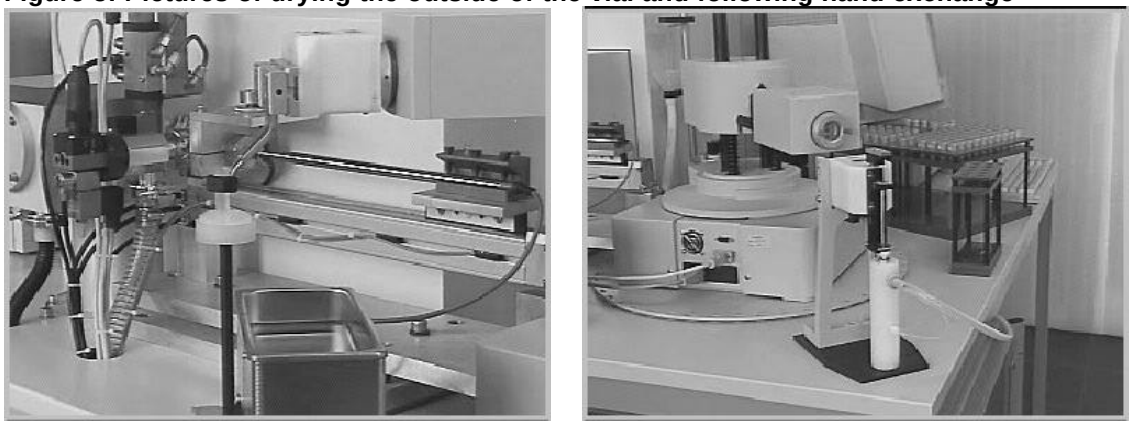


Figure 6: Filling the syringe with sample and moving it to sample transfer position

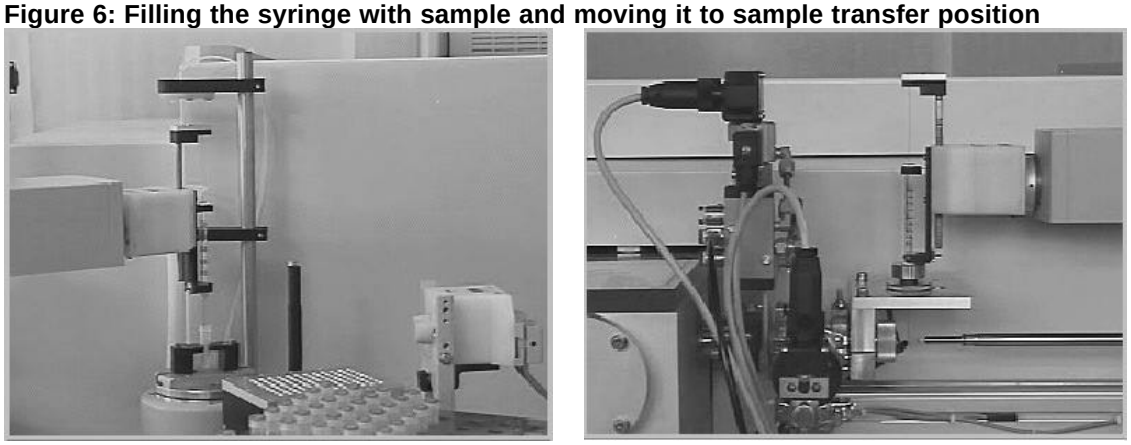
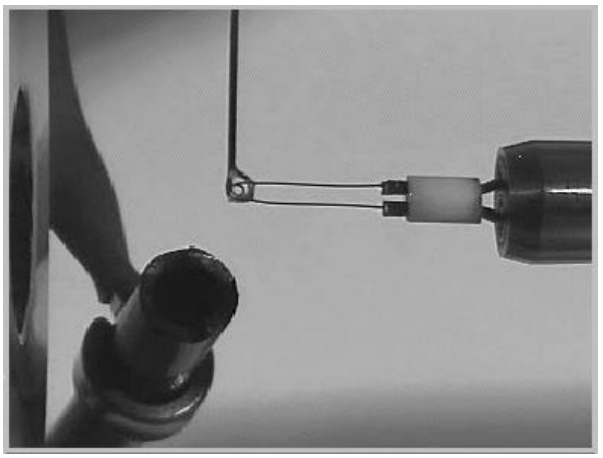


Figure 7: Sample transfer from syringe to DCI-wire



AUTOMATED DCI-MASS SPECTROMETRIC ANALYSIS

30 seconds after sample transfer (Figure 7) the solvent is evaporated and the DCI-wire, which is mounted on a probe, is ready to be inserted to the vacuum region of the mass spectrometer. The micro processor controlled sample introduction system (Figure 8) moves the probe to the fore-vacuum lock and after having evacuated the lock, it continues to the CI-ion source in the high vacuum region (Figure 9). Now the filament and high voltage are switched-on and the mass spectrometer starts data acquisition. After a 5 sec. delay the DCI-wire is being heated according to a 35 sec. linear current-ramp. The DCI process begins and the mass spectrometer acquires a series of approx. 86 scans.

Figure 8: Micro processor controlled sample introduction system

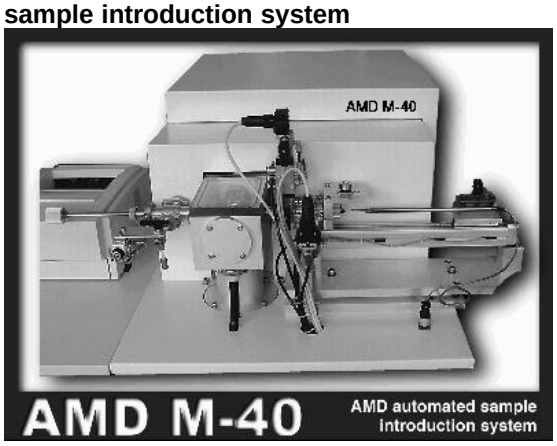
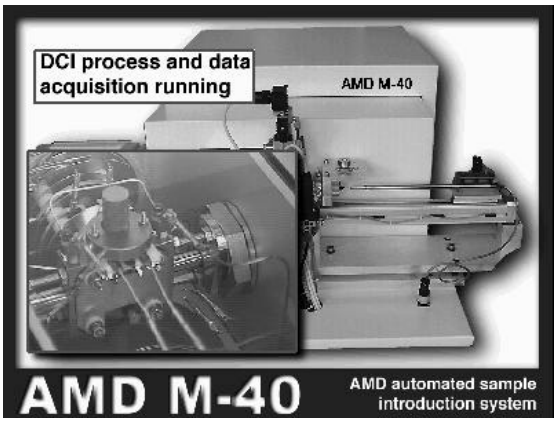


Figure 9: Ion Source



AUTOMATED DATA EVALUATION

In Figure 10 a typical DCI desorption curve is presented. The curve represents the total ion current (TIC) during the desorption process. Similar to chromatograms in GC/MS or LC/MS analyses the most characteristic spectrum of the substance is contained within this „peak“ of the TIC trace.

The last step of the procedure is the data evaluation which is automatically performed, too. The program algorithm selects the most specific mass spectrum (Figure 11) from the TIC trace. Besides other statistical parameters the expected molecular weights play a significant role for the identification of the spectrum.

Figure 10: Typical desorption curve of the total ion current

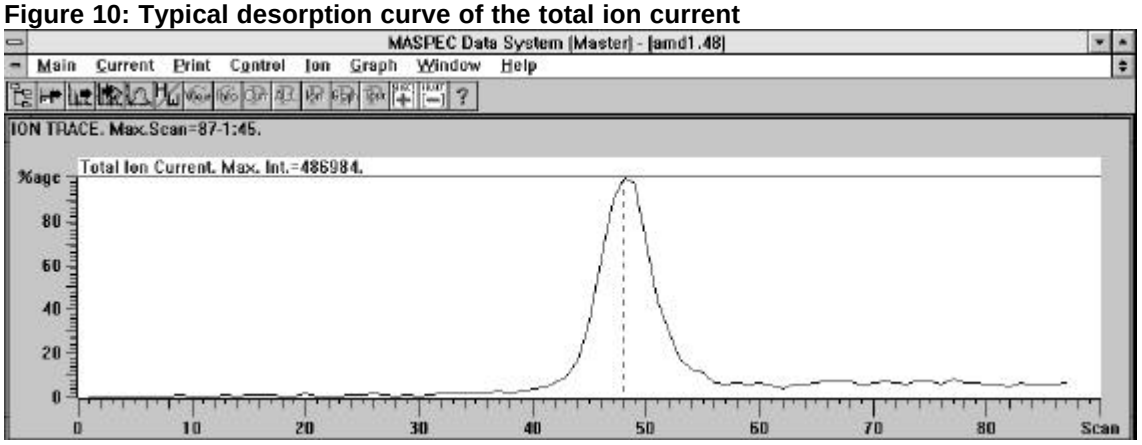
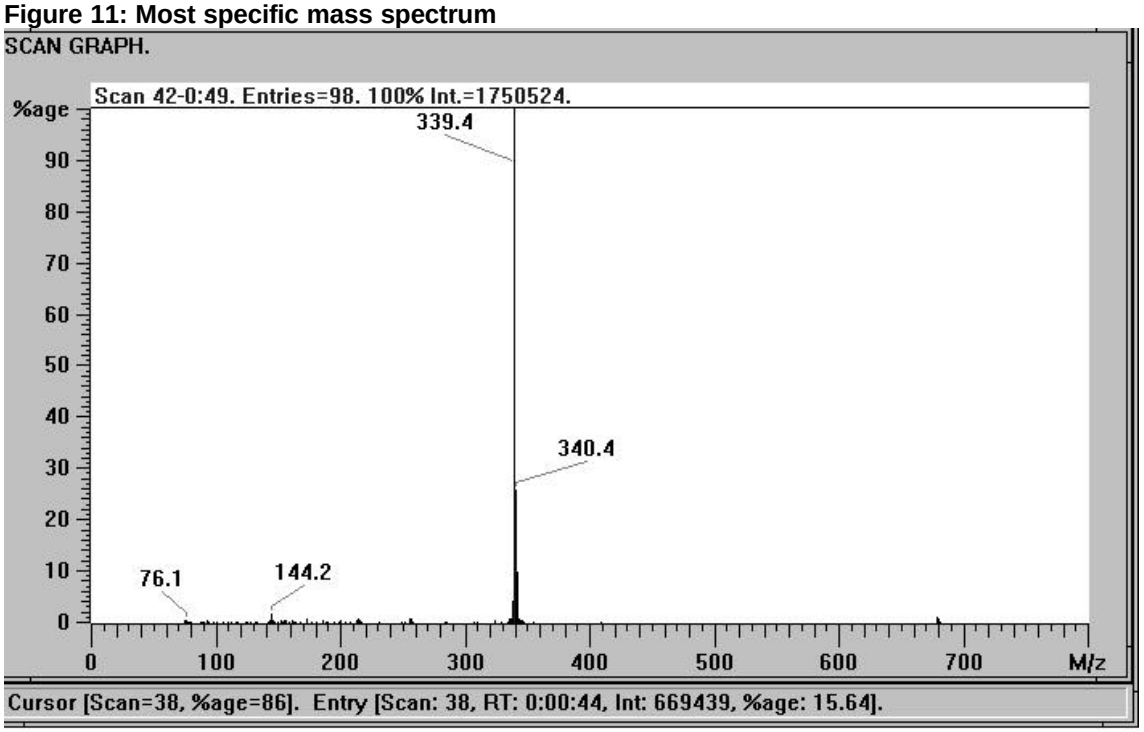


Figure 11: Most specific mass spectrum



In addition to the spectrum, the automatically printed report contains all required sample information which were already filled in the Excel spreadsheet at the Zymate™ computer at the very beginning of the procedure.

High Sample Throughput

The whole cycle, sample preparation of solid samples and mass spectrometric analysis, takes about four minutes. Therefore, within eight hours 120 samples can be automatically analyzed during the day and another 120 samples can be

measured during the night. Compared to the manually performed analyses the sample throughput could be increased by a factor of six with significant decreased man power at the same time.

LC/MS COUPLING

AMD Intectra has developed a unique **Combination API/EI-Ion Source** which includes API ionization techniques (Electrospray, Atmospheric Pressure Chemical Ionization) which are well suited for LC/MS coupling. The ions produced under atmospheric pressure are being transferred to the EI ion source and therefore, API and EI/CI analyses can be performed without any system modification.

Fully automated LC/MS or ESI-MS analyses can be run on the AMD M-40 series of mass spectrometers in a similar way as described for the DCI-analyses.

REFERENCES

M. E. Rose and R. A. W. Johnstone, „Mass spectrometry for chemists and bio-chemists“, Cambridge University Press 1982, U.K., pp. 120-126.

M. A. Baldwin, F. W. McLafferty: Org. Mass Spectrom. 7, 1353 (1973).

TRADEMARKS