$R_1 = 0.0778$, wR_2 $(F^2) = 0.1857$. This structure was solved by direct methods and refined by full-matrix least squares on F^2 by using the Siemens SHELXTL/PC package of crystallographic software.[18] All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were geometrically fixed using the riding model. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-112284. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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ESI Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR-MS): A Rapid High-Resolution Analytical Method for Combinatorial Compound Libraries**

Tilmann B. Walk, Axel W. Trautwein, Hartmut Richter, and Günther Jung*

Test systems for the search for active materials are largely automated and designed for the reliable recognition of in vitro activity with minimal sample consumption (high-throughput screening). Compound collections for mass screening are nowadays prepared by the methods of combinatorial chemistry.[1, 2] The parallel synthesis of hundreds and thousands of compounds per day is state of the art (high-throughput synthesis). The requirement for rapid characterization of the compounds (high-throughput analysis) arises from this development. An increase in the efficiency of "off-bead" analysis by ESI/MALDI-TOF/quadrupole-MS[3, 4] and reverse-phase HPLC[5] as well as "on-bead" analysis by NMR[6] and FT-IR spectroscopy[7] has been rapidly achieved. Previously less utilized methods such as FT-IR microscopy even allow simultaneous "on-bead" analysis of polymer-bound compound collections on hundreds of resin beads.[8]

FT-ICR mass spectrometry[9] sets new standards with respect to effectiveness, informative value, and sample consumption. In Figure 1, measurements obtained by ESI-quadrupole (A) and ESI-FT-ICR mass spectrometry (B) on a compound prepared by solid-phase organic synthesis[10] are superimposed. The ESI-FT-ICR measurement gives a mass accuracy which is greater by a factor of about 550 (A: $\delta = 221$ ppm; B: $\delta = 0.4$ ppm). Accordingly, the difference between calculated and measured mass of the FT-ICR determination corresponds to 0.00018 Da in this example. The resolution is about a factor of 15 better than for the comparable measurement with a quadrupole analyzer (A: 4500; B: 70000). The FT-ICR measurement was taken in the broad-band mode with a scanned mass range of 1400 Da. This measurement mode is not designed for the highest resolutions, but nevertheless a resolution of 70000 was obtained. The corresponding measurement in the high-resolution mode at a scanned mass range of 100 Da gave a resolution of 350000 (data not shown).

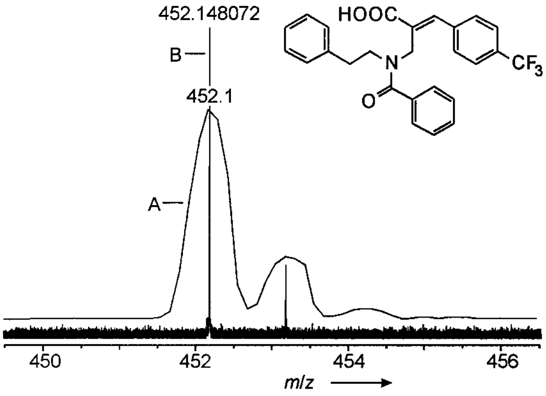

Figure 1. Superimposed measurements of a compound obtained by solid-phase synthesis with ESI-quadrupole (A) and ESI-FT-ICR mass spectrometry (B). Starting points for elemental analysis: $[M-\mathrm{H}]^-$ measured: 452.1480720 Da, charge −1, min./max. double bond equivalents (DBE) 0.5/30, tolerance ±0.0005 Da. Elemental analysis for $M_r = 452.1480720$: C 26, O 3, N 1, S 0, H 21, F 3; $[M-\mathrm{H}]^-$ calculated: 452.1478911, 15.5 DBE, deviation 0.4 ppm. The calculation of possible molecular formulas on the basis of the very precise ESI-FT-ICR measurement thus gave solely the correct composition.

The amount of sample required for an FT-ICR measurement is 10 times less than for the quadrupole measurement (A: 40 pmol; B: 4 pmol). The tolerance of the FT-ICR measurement resulting from the mean accuracy of the

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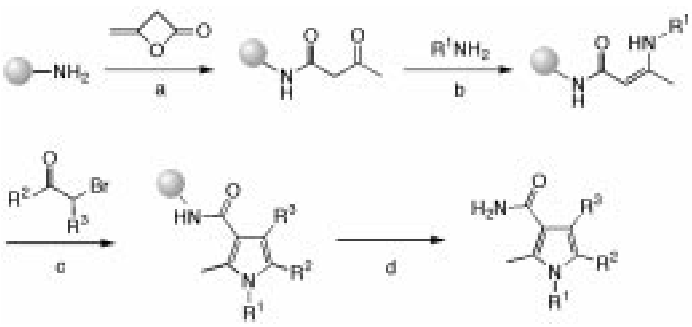
measurements is very small (0.0005 Da), for which reason the number of postulated molecular formulae is small. Owing to the high resolution, the isotope peaks are especially easy to recognize. When the exclusion rules are taken into consideration (nitrogen rule, double bond rule, valences), the calculation for this example gives solely the elemental composition which corresponds to the actual molecular formula. The FT-ICR measurement thus enables rapid elementary analysis[11] of individual compounds in the pharmacologically interesting range of up to 500 Da.

This rapid molecular formula determination may also be applied to complex mixtures such as those which arise from combinatorial chemistry. To demonstrate the performance of this method in this respect, a split–mix compound collection of 140 different pyrrole amides consisting of 10 subcollections of 14 compounds each was synthesized on Rink amide AM resin (Scheme 1).[12]



Scheme 1. Solid-phase synthesis of pyrrole amides by the split–mix method. a) Acetoacetylation with diketene; b) separation of the resin into 14 aliquots, enaminone formation with 14 different primary amines; c) combination of the 14 aliquots, separation into 10 aliquots, reaction with 10 different α-bromoketones; d) cleavage from the polymer support.

The results of the subcollection with 2-bromoacetophenone as building block ($R^2 = C_6H_5$, $R^3 = H$) are illustrated as an example. The total amount of sample of 20 pmol gives a mean sample consumption of 1.4 pmol per compound for the routine measurement. All 14 expected products could be detected by mass spectrometry (Figure 2).

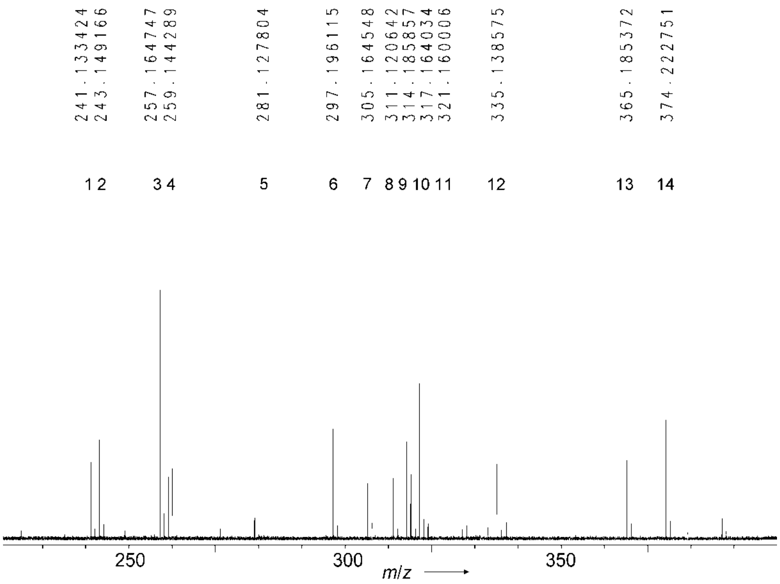


Figure 2. Automated routine ESI-FT-ICR measurement of the pyrrole amide collection (section from m/z 220–400). The duration of the measurement was about 30 s; the measurement range of 1400 m/z was measured 16 times during this time. Processing of the data collected (selection of smoothing parameters and number of data points, peak labeling) was automated.

The resolution averaged over all 14 compounds ($m/\Delta m_{50\%}$) is 38000. The mean mass accuracy Δm is 0.00032 Da (1.05 ppm) at a standard deviation of 0.00026 Da. The possible molecular formulas were calculated for all 14 masses of the measurement. The exact masses for the elements C, H, N, O, and S were considered by the program, and results with a nitrogen content of over 50 weight percent were rejected (Table 1). The very high mass accuracy allows only one or two, at the most three, possible elementary compositions (hits) for each compound. The individual compounds are thus so precisely characterized that, with prior knowledge of the reaction conditions, the structural formulae may also be determined.

Table 1. Results of the ESI-FT-ICR measurement illustrated in Figure 3. The high mass accuracy (mean value $\delta = 1.05$) allows only one or two, at the most three, possible molecular compositions (hits) per compound.

Compd	Amine (R^1)	Molecular formula	Mass calcd $[M+H]^+$	Mass found $[M+H]^+$	Δ mass	δ	Hits
1	cyclopropylamine	$C_{15}H_{16}N_2O$	241.133530	241.133424	0.000106	0.44	1
2	isopropylamine	$C_{15}H_{18}N_2O$	243.149179	243.149166	0.000013	0.05	1
3	1-butylamine	$C_{16}H_{20}N_2O$	257.164829	257.164747	0.000082	0.31	1
4	3-amino-1-propanol	$C_{15}H_{18}N_2O_2$	259.144093	259.144289	0.000196	0.75	2
5	furfurylamine	$C_{17}H_{16}N_2O_2$	281.128444	281.127804	0.000640	2.27	2
6	aminomethylcyclohexane	$C_{19}H_{24}N_2O$	297.196127	297.196115	0.000012	0.04	1
7	2-phenylethylamine	$C_{20}H_{20}N_2O$	305.164829	305.164548	0.000281	0.92	2
8	2-(2-aminoethyl)thiophene	$C_{18}H_{18}N_2OS$	311.121294	311.120642	0.000652	2.09	3
9	*N*-(2-aminoethyl)morpholine	$C_{18}H_{23}N_3O_2$	314.186290	314.185857	0.000433	1.37	1
10	1-aminoindane	$C_{21}H_{20}N_2O$	317.164829	317.164034	0.000795	2.50	2
11	tyramine	$C_{20}H_{20}N_2O_2$	321.159743	321.160006	0.000263	0.81	2
12	piperonylamine	$C_{20}H_{18}N_2O_3$	335.139008	335.138575	0.000433	1.29	2
13	3,4-dimethoxyphenylethylamine	$C_{22}H_{24}N_2O_3$	365.185955	365.185372	0.000583	1.59	2
14	4-amino-*N*-benzylpiperidine	$C_{24}H_{27}N_3O$	374.222674	374.222751	0.000077	0.20	2
				mean value:	0.000326	1.05	1.57
				standard deviation:	0.000264	0.83	

The very high accuracy and resolution of the measurements alone represent a considerable increase in the efficiency of mass spectrometric methods. The precise separation by mass of the direct measurement of a compound mixture frequently avoids prior separation by HPLC. As the result of direct determination, time-consuming and expensive tagging systems for compound mixtures of up to 100 components become unnecessary. The time saved even for low sample availability is considerable.

To meet the demands of high-throughput analysis, we automated the sample input. For this purpose an HPLC system was coupled directly to the FT-ICR mass spectrometer. The integrated automatic sample injector inputs a new sample for measurement into the FT-ICR mass spectrometer by flow injection every two minutes. Together with the synchronized start of the FT-ICR measurement, this allows a sample throughput of 300 hundred samples per night. At the end of the measurement the raw data collected are processed automatically according to the wishes of the user (number of data points, choice of smoothing parameters, peak labeling, etc.). After the automatic sample injector is reset the mass spectrometer is ready for use.

Mass spectrometric characterization of isomers requires the coupling of HPLC and mass spectrometer. The results of HPLC/FT-ICR-MS analysis of the aforementioned subcollection is illustrated in Figure 3 as a contour diagram.

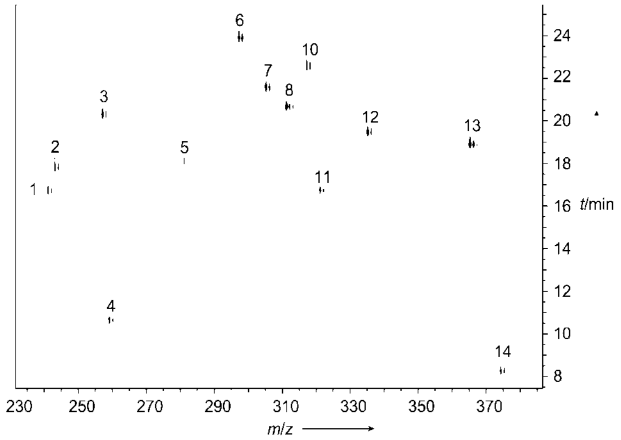


Figure 3. Contour diagram of the HPLC/FT-ICR-MS coupling of the pyrrole amide compound collection with $R^2 = C_6H_5$, $R^3 = H$. Isomers with the inherent separation problems could also be detected with this method. The isotope resolution of the molecule ion peaks is readily seen.

The measurement described utilizes fully all technical possibilities for improving the performance of mass spectrometric methods for use in high-throughput analysis. These are currently the best possible resolution and mass accuracy over a broad mass range, short measurement times, lowest sample consumptions, and automated sampling and processing. This method is thus an important aid in synthesis optimization and control of individual compounds and compound collections in combinatorial chemistry. The system is also ideally suited for use in protein analysis, for the elucidation of natural products by MS^n experiments, or for metabolic studies.

Experimental Section

The measurements were carried out with an Apex II-ESI-FT-ICR mass spectrometer (4.7 T) from Bruker Daltonik. The number of data points per measurement was 256 K in a mass range of 100–1500 Da. The mass range was scanned once a second, and 16 scans were taken. The ESI-FT-ICR-MS and automatic sampler were coupled through a fused silica capillary (75 µm internal diameter, 365 µm external diameter). The samples were injected into the continuous solvent stream (flow rate 30 $\mu L\,min^{-1}$, 60% acetonitrile, 40% water, each with 0.1% formic acid) by the automatic sample injector of the HPLC system (Hewlett Packard, HP-1100 series). Measurements with a known compound collection provided the data for three-point calibration of the mass spectrometer. The subcollection for the HPLC/FT-ICR-MS coupling was separated on an RP-C 18 column (GROM-Sil ODS7; 1 × 6 mm; gradient of 10–80% acetonitrile/water plus 0.1% formic acid in 25 min at a flow rate of 50 $\mu L\,min^{-1}$).

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