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High-throughput flow injection analysis–mass spectrometry with networked delivery of colour rendered results: the characterisation of liquid chromatography fractions

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Abstract

An automated high-throughput flow injection analysis electrospray–mass spectrometry system originally developed in-house for purity estimation of multiple parallel synthesis combinatorial chemistry samples, was adapted to analyse liquid chromatography fractions. A networked Visual Basic application called RACKVIEWER provides a fast and easy inspection of colour rendered liquid chromatography fraction data, by the synthetic chemists, whether at the main corporate site or the various geographically dispersed Novartis Pharma research institutes. Various depictions of individual well ion currents including colourised three-dimensional maps of the rack's entire ion current, provide data highlighting strategies. Other advantages include allowing the seamless integration of 96-well racks containing the liquid chromatography fractions into existing quick turnaround high-throughput mass spectrometry queues, and allowing the synthetic chemists a wider a priori choice of MS compatible chromatographic conditions.

Keywords: Flow injection analysis; Mass spectrometry; Detection, LC

1. Introduction

Mass spectrometry (MS) facilities in pharmaceutical companies have been generally forced to increase their sample processing rate to accommodate the development of industrial scale high-throughput synthesis and screening. This forces the delegation of some MS operator decisions to automatic systems [1].

Over the last 3 years, the MS section in Novartis Pharma Research has analysed tens of thousands of multiple parallel synthesis samples, using an in-house automated high-throughput flow injection analysis mass spectrometry (FIA–MS) system. The primary goal of this was to deliver the consequent purity estimates in a quick and clear way to geographically dispersed combinatorial chemistry laboratories; here purity is defined as the sum of the ion current in a spectrum ascribable to the expected compound, including the adducts, divided by the spectrum's total ion current. The combinatorial chemists then combine these with their LC–UV purity estimates to judge the success of their synthesis. Colour rendering of the MS data within a Visual Basic graphic interface program was used [2,3], with numerical purities relegated to the (accessible) background. Four different primary displays

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of the MS data were made accessible via single mouse-clicks (Fig. 1); switches exist within two of these for calling different colourisations.

One persistent theme for us is how to efficiently match mass spectrometer time to the priority and complexity of samples. An illustration of this was the need for the MS section to accommodate some samples for on-line liquid chromatography–mass spectrometry (LC–MS) on the high-throughput mass spectrometers, given the delays involved in preparation and optimisation of liquid chromatographic conditions. An associated problem, in contrast to the FIA–MS of the combinatorial chemistry samples, was the subsequent delivery of LC–MS results to the synthetic chemists. This is due to the lack of systems that can automatically and comprehensively process eluting chromatographic peaks in electrospray LC runs [4] and report easily interpretable results over networks to the synthetic chemists at their work-benches. A LC–MS system involving networked reporting of numerical tables of standardised LC runs possessing well-conditioned UV traces has been reported [5].

Therefore, as a first step, it was decided to adapt our FIA–MS system for LC fraction analysis. This has certain advantages. Firstly and most importantly, the synthetic chemists normally get MS results back within 24 h at their work-bench personal computers; they can then decide if a more resource intensive on-line LC–MS analysis is needed. As the synthetic chemists have usually more sample information than the mass spectrometrist they can thus allocate their

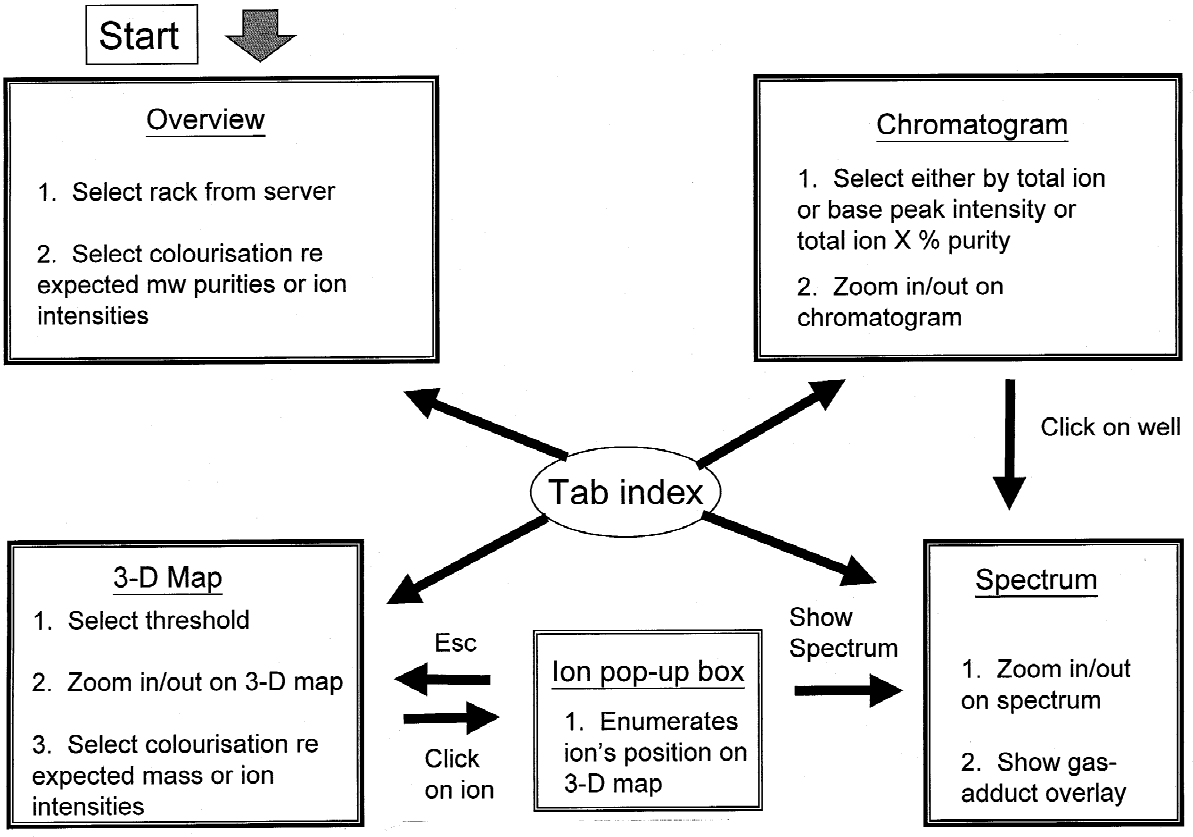


Fig. 1. RACKVIEWER routemap, showing the links between the four main displays. The chemists directly jump into the purity Overview, upon accessing their racks on the central server. From Overview, only one mouse-click is required to access the Spectra, Chromatogram and 3D map displays.

budgets for analytics in a more proportional manner. For instance, LC–UV results are often available for industrial synthetic samples prior to their delivery to MS analysis. Secondly, the synthetic chemists have a wider a priori choice of MS compatible chromatographic conditions [5] e.g. FIA–MS is more resistant than LC–MS to non-volatile buffers. Thirdly, for ease of use the results are rendered by colours rather than via numbers. Finally, the synthetic chemists browsing through their results do not necessitate the fast transfer of very large LC–MS data files from a central server i.e. LC fraction data is not an excessive burden on networking resources. One disadvantage of using LC fractions rather than conventional coupled LC–MS is the reduced chromatographic resolution and sensitivity. However, sensitivity is rarely an issue as low milligram amounts are normally available even at the very early stages in industrial synthetic chemistry.

Our original high-throughput FIA–MS system [2], designed for purity estimation of combinatorial chemistry samples has some similarities with an earlier report [6] and with the automated open access mass spectrometers analysing conventional synthetic chemistry samples [7]. However, the popular ionisation mode for open access MS is atmospheric pressure chemical ionisation (APcI) rather than electrospray ionisation (ESI) [8]. This is because APcI is a harder ionisation mode than ESI, with less adduction and clustering [9]. Consequently, APcI is more likely to generate a quasimolecular ion less obscured by adduction, which facilitates spectral interpretation for the synthetic chemists [10]. However, for the (spectral) purity estimation of samples with expected formulae, the adduction prevalent in ESI is preferable to the risk of unanticipated fragmentation induced by APcI, especially as the large sample numbers effectively prevent comprehensive manual inspection of the spectra. Consequently, the use of intentional up-front or in-source collision-induced dissociation (CID) using a voltage offset is also avoided.

To illustrate the various new features of RACKVIEWER created to accommodate the FIA–MS of LC fractions, an entire semipreparative LC fractionation from a combinatorial chemistry synthetic laboratory was measured by both positive and negative ion electrospray FIA–MS.

2. Experimental

2.1. Combinatorial chemistry synthesis

The combinatorial chemistry compound of interest was an N-terminally acylated tripeptoid analogue with oligo (N-substituted glycine) backbone and arylalkyl groups and an aliphatic carboxylic acid as nitrogen substituents. The expected compound had a stoichiometric formula $C_{31}H_{42}N_4O_6$, with a monoisotopic molecular mass of 566.3. Due to the fully substituted nitrogens and the carboxylic group, the compound is detectable in both the positive and negative ion mode.

The compound was synthesized at the 140 μmol scale by the submonomer method [11] on a polystyrene solid support with Rink amide linker by reacting consecutively bromoacetic acid and amine components. After acylation, the crude product was cleaved by two 45-min treatments with TFA–methylenechloride (1:1, v/v) containing 2% triisopropylsilane, evaporated to dryness and then dissolved in acetonitrile–water (7:3, v/v). From 110 mg crude product, a 2-mg aliquot was separated by LC for subsequent MS analysis. After identification of the desired product peak, the bulk material was purified by LC to yield 35 mg, i.e. 44% yield.

2.2. Combinatorial chemistry LC system

The solvent delivery system consisted of a Waters 590 HPLC pump and a low pressure gradient mixer with two Bürkert valves and a custom-made controller. A Waters 8×10 radial compression module with a 100×8 mm Prep NovaPak HRC_{18} 6 μm 60 Å cartridge and a 4×4 mm Merck RP18 SelectB precolumn was used. The detector was a LKB Uvicord SII with a 227 nm filter, 8 μl cuvette and 2.5 mm pathlength. Data acquisition was with a v4.22 GYNKOSOFT chromatography data system. LiChrosolv gradient grade acetonitrile, LiChrosolv 'water for chromatography' and analytical grade trifluoroacetic acid (TFA) from Merck (Darmstadt, Germany) were used. The sample was separated with a gradient from 35 to 70% solvent A in 20 min, then isocratically at 70% solvent A for 10 min. Solvent A was acetonitrile–water–TFA (5:95:0.1, v/v/v) and solvent B was acetonitrile–water–TFA (95:5:0.1,

v/v/v). The flow-rate was 1.8 ml/min. In total, 84 fractions each consisting of 0.6 ml, representing 0.33-min intervals, were collected into a Pharmacia Frac100 fraction collector with inserts holding Micronic 45×8.8 mm tubes.

The tubes were placed in a 96-tube holder and evaporated to dryness in a GeneVac Atlas HT-4 vacuum centrifuge evaporator (GeneVac, Ipswich IP15AP, UK), then placed in a Micronic rack in positions B1-H12, sealed and express posted to Novartis Pharma, Basel, Switzerland. The rack identification, sample positions and expected molecular masses were electronically sent to a computer drive on a corporate server in Basel, as an Excel CSV format file.

2.3. Rack delivery and preparation

In parallel with their physical delivery to the MS laboratory, Microsoft Excel CSV format files describing each rack are deposited on a central server by the synthetic chemists. The Excel files are then called by the MS operator and UNIX script files then automatically build the ICIS analysis page methods. This involves the individual synthetic chemist's name, rack's name, desired ionisation mode, location of occupied wells with their formulae and calculation of expected monoisotopic molecular mass and adduct m/z positions.

The FIA–MS system [2,3] uses the 96-well Micronic sample rack as the basic sample handling unit. This rack was also adopted as the standard handling unit for LC fractions, as these can be both seamlessly integrated into the concatenated methods lists in the Finnigan ICIS Analysis page which constitute the sample queue and into the physical queue positions on the multirack autosampler. The LC fraction racks can be inserted anywhere in the 24-h queues that exist on the two mass spectrometers dedicated for high-throughput analysis. These FIA–MS queues are dominated by 96-well racks from combinatorial chemistry with expected molecular masses; the default ionisation method for LC fraction racks is therefore ESI. The measurement duty cycle for each LC fraction is 90 s.

Each LC fraction rack has a yohimbine standard curve installed in row A by the MS operator, for the sake of maintaining inter-rack comparability. This was made from yohimbine hydrochloride (Sigma part no. Y-3125), dissolved in 1.0 ml acetonitrile–water (70:30, v/v). Wells A1, A11 and A12 are blanks containing only acetonitrile–water (70:30, v/v). Wells A2 to A10 represent 1, 5, 10, 50, 100 and 500 μg/ml, and 1, 5 and 10 mg/ml. In the negative ion mode and positive ion mode the standard curve saturates above 500 and 50 μg/ml, respectively.

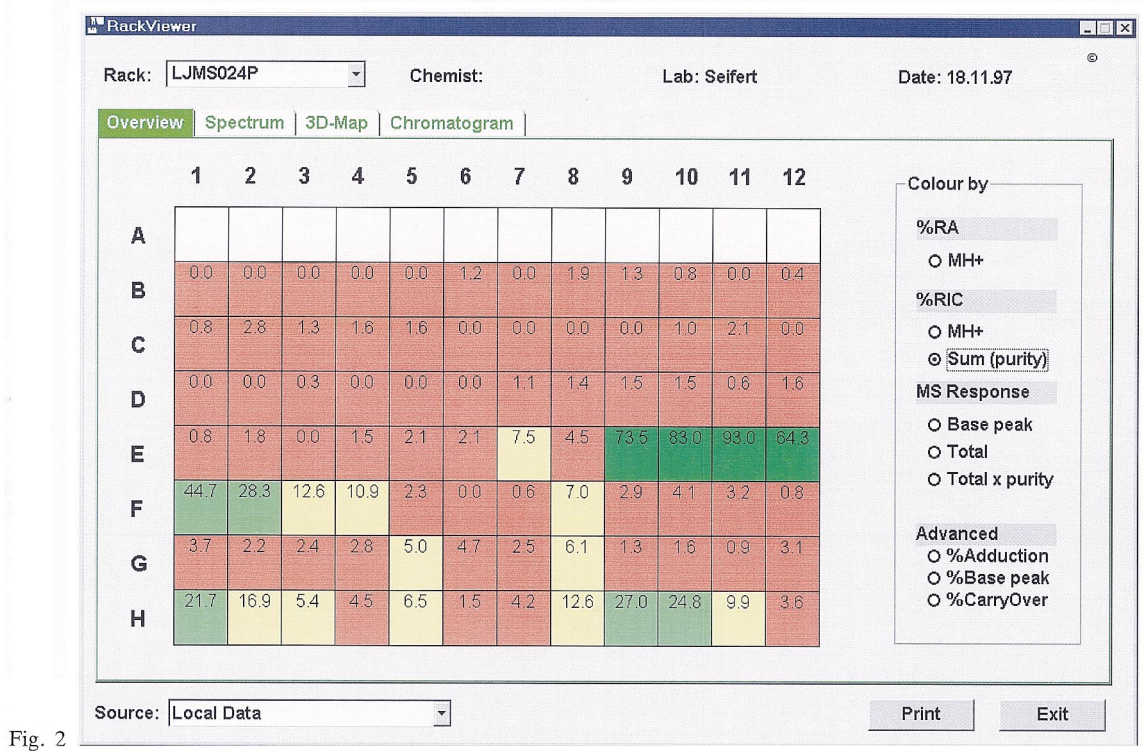
For LC fraction racks, the synthetic chemists often supply no expected stoichiometric formula in the accompanying Microsoft Excel CSV files. In this case, the MS operator modifies the CSV file so that the purity estimation defaults to reporting yohimbine purities for the entire rack, not just for the yohimbine standard curve inserted by the MS operator in row A. If a formula is supplied by the synthetic chemists, then yohimbine purities are reported only for the standard curve in row A. Colourisation then occurs for both expected monoisotopic molecular mass (Fig. 2) and ion currents, in the remaining rows. One consequence of the frequent absence of expected molecular masses in submitted fraction sets is that the centroid acquisition scan range is no longer automatically allocated, dependent on molecular mass but standardised at 160 to 1560 m/z per 2 s.

2.4. Mass spectrometry.

The MS operating conditions in ESI positive and negative modes were: heated capillary temperature, 220°C; conversion dynode at 15 kV; electron multiplier at 0.9 kV, a collision-induced dissociation offset

Fig. 2. Overview with positive ion ESI purity colourisation, according to an expected formula $C_{31}H_{42}N_4O_6$ with a monoisotopic molecular mass of 566.3. This shows immediately that the expected compound occurs in fractions E9 to F2. A convention is that the synthetic chemists leave free the first row. In this experiment it was occupied by a yohimbine standard curve (not shown). A false positive at well H1 is caused by a compound with a monoisotopic molecular mass of 584.3 being interpreted as the $[M+H+CH_3CN]^+$ adduct of the expected compound. Wells were measured in a left to right and top to bottom sequence i.e. B1 to B12.... H1 to H12, which parallels the LC elution order.

Fig. 3. 3D map of positive ion ESI spectra, with no normalisation to expected monoisotopic molecular mass and colourised to % RA (intensity). Horizontal tracks of red colourised ions less than nine wells wide i.e. 3 min, indicate probable quasimolecular ions.


RackViewer
Rack: LJMS024P
Chemist:
Lab: Seifert
Date: 18.11.97
Overview
Spectrum
3D-Map
Chromatogram
1 2 3 4 5 6 7 8 9 10 11 12
A 0.0 0.0 0.0 0.0 0.0 1.2 0.0 1.9 1.3 0.8 0.0 0.4
B 0.8 2.8 1.3 1.6 1.6 0.0 0.0 0.0 0.0 1.0 2.1 0.0
C 0.0 0.0 0.3 0.0 0.0 0.0 1.1 1.4 1.5 1.5 0.6 1.6
D 0.8 1.8 0.0 1.5 2.1 2.1 7.5 4.5 73.5 83.0 98.0 64.3
E 44.7 28.3 12.6 10.9 2.3 0.0 0.6 7.0 2.9 4.1 3.2 0.8
F 3.7 2.2 2.4 2.8 5.0 4.7 2.5 6.1 1.3 1.6 0.9 3.1
G 21.7 16.9 5.4 4.5 6.5 1.5 4.2 12.6 27.0 24.8 9.9 3.6
H
Colour by
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MH+
Sum (purity)
MS Response
Base peak
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Total x purity
Advanced
%Adduction
%Base peak
%CarryOver
Source: Local Data
Print
Exit

Fig. 2

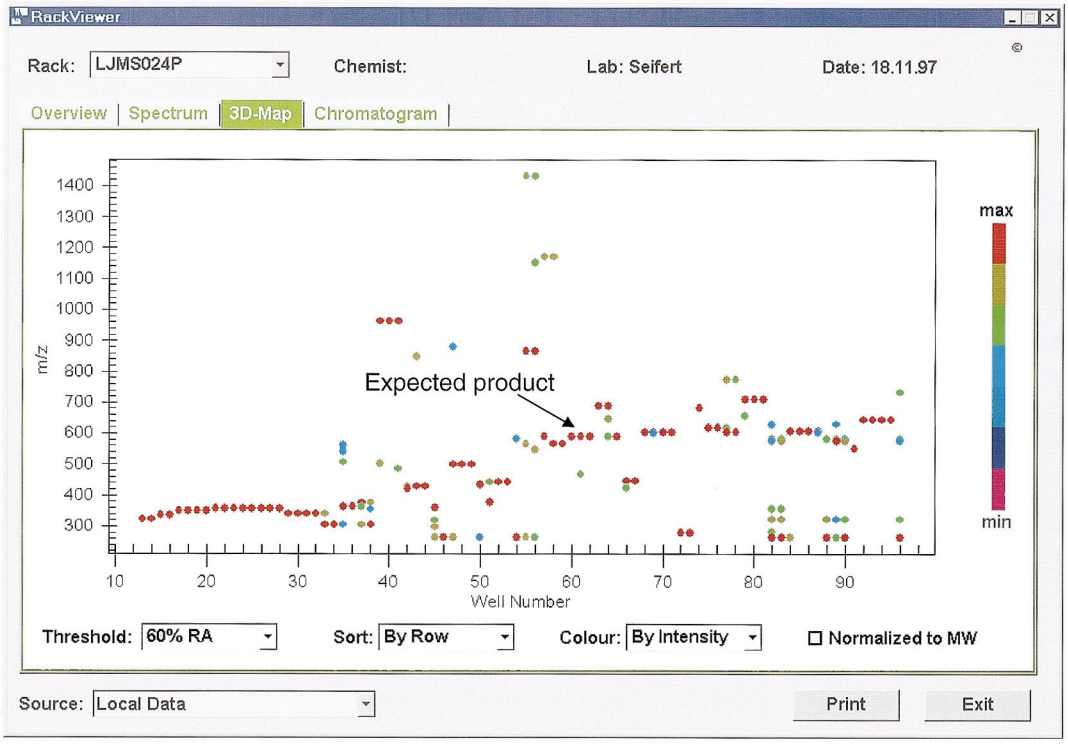
RackViewer
Rack: LJMS024P
Chemist:
Lab: Seifert
Date: 18.11.97
Overview
Spectrum
3D-Map
Chromatogram
m/z
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
Expected product
10 20 30 40 50 60 70 80 90
Well Number
max
min
Threshold: 60% RA
Sort: By Row
Colour: By Intensity
Normalized to MW
Source: Local Data
Print
Exit

Fig. 3

of 0.0 V and a spray voltage 4.5 kV. The FIA solvent was acetonitrile–water (70:30, v/v), using LiChrosolv gradient grade acetonitrile and LiChrosolv 'water for chromatography' from Merck. The FIA flow-rate was 50 μl/min. Normally 2 μl of sample was injected, onto a 10-μl loop. A 55-cm long polyether ether ketone (PEEK) tubing 0.005 in. I.D.×1/16 in. O.D. (Upchurch Scientific, part no. 1535) linked via a zero-dead volume union with a 40-cm long ESI fused-silica capillary 200 μm O.D.× 100 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA part no. TSP100200), joined the autosampler Valco valve to the exit end of the electrospray spray nozzle (Finnigan part no. 70005-20169) [1,2]. The head pressure for the ESI source nitrogen sheath gas was 60 p.s.i. The Finnigan API interface version no. is characterised by the dimensions of the source heated capillary i.e. 0.016±0.001 in. I.D.×1/16 in. O.D. (Finnigan part no. 70005-98038).

A Finnigan MAT SSQ-7000 mass spectrometer (San Jose, USA) was used in these experiments, controlled by a DEC AXP 3000/300 workstation under the OSF/1 V3.2D operating system running the Finnigan interactive chemical information system (ICIS) V8.2.1 application software. It was twinned with a physically adjacent SSQ-7000. Two Ethernet-interfaces connected each workstation to a SSQ-7000 and the Novartis local area network (LAN), respectively. Both workstations shared a common disk area as a network file system. A dedicated file service accessible by the synthetic chemists was set up on the Novartis LAN, to exchange files with that area via TCP/IP (Samba).

The Finnigan ICIS package controls a Hewlett-Packard 1090 HPLC through a HP-IB interface and the autosampler via the RS-232C interface. An autosampler with capacity for eight 96-well racks was used (model HTS PAL from CTC Analytics, Zwingen, Switzerland).

The initial CTC installation software (v1.3.108) was replaced by a later version (v1.3.135) enabling the six ports Valco valve inject/load orientation to be verified prior to every injection. The 96-well Micronic Blueline racks were from Micronic (Lelystad, The Netherlands). Samples are usually measured in a left to right sequence, B1 to B12, C1 to C12,.... H1 to H12.

The illustrated 96-well Micronic rack was received by post from Vienna. The evaporated residue in each tube was redissolved in acetonitrile–water (70:30, v/v). The RACKVIEWER application was developed using Microsoft Visual Basic V5.0 for the 32-bit environment of Microsoft Windows NT. All the *x–y* graphics in RACKVIEWER e.g., spectra and three-dimensional (3D) map, are built from peak lists using a commercial graphics control (PROESSENTIALS from Gigasoft (Keller, TX, USA).

3. Results and discussion

3.1. General changes

Two displays existed in the first version of RACKVIEWER [2] i.e., Overview and Spectrum (then called Sample). A 3D map display was added in the second version for enhancement of the combinatorial chemistry aspects [3] and a Chromatogram display was added to the third (current) RACKVIEWER version, to adapt it for LC fraction analysis, Fig. 1. The RACKVIEWER application presently has several advantages over commercially available 96-well rack browser software from the major mass spectrometer manufacturers. Firstly it has a greater diversity of colourisation switches, including ion currents e.g. total ion current multiplied by percentage purity. Secondly it has both 3D map and histogram displays, with zoom in and out facilities.

In the first RACKVIEWER version [2], spectra were thresholded at 5% relative abundance (% RA). Now a peak list of the automatically background subtracted spectrum i.e. *m/z*, % RA, thresholded at 1% is used for the purity calculations. Thresholding is a critical parameter and if set too low e.g., 0.1% could cause slowness in graphical display of data over the network, and if set too high can cause inaccuracies in the purity calculations.

Zoom in and zoom out is provided for three of the four RACKVIEWER displays i.e. Spectrum, 3D Map and Chromatogram. These three may be exported to other Windows applications via the clipboard. Printing facilities are available via a Print button for all four displays.

3.2. Overview display

The existing colourisation switch originally designed for combinatorial chemistry purity estimation

was used to scan the complete fraction set for the expected synthetic product, Fig. 2. To adapt the combinatorial chemistry colourisation schemes of the original RACKVIEWER for LC fractions, several new 'MS response' colourisation switches were developed for the Overview display.

The first new Overview (not shown), representing the total ion current counts of the spectrum in every well, was colourised according to a 16-colour rainbow scheme. A vertical minimum/maximum colour bar legend showing the various colours appears when either of these two displays are activated. The second new Overview (not shown) representing the ion current of the base peak, i.e. the largest ion of the spectrum in every well, was also similarly rainbow colourised.

The third new Overview (not shown) represents the total ion current counts of the spectrum in every well, multiplied by the % purity. This was again colourised to a 16-colour rainbow scheme. This is especially useful for pinpointing the fraction with the greatest amount of expected compound(s), when dealing with broad chromatographic peaks e.g., from semipreparative LC columns, each displaying high % purities. In these three Overview colourisations, the $\log_{10}$ of the ion current counts are numerically shown inside the well quadrant.

3.3. Three-dimensional map display

Another display was developed called the 3D map. This presents a bird's eye view of an entire LC fraction MS data set using *X* axis (fraction no.), *Y* axis (m/z) and colour [3]. This view has some similarities to the report [12] which used *X*, *Y* and *Z* axes for depiction. The ions are colourised according to % RA in a seven-colour gradation, with a vertical minimum/maximum colour bar legend adjacent to the 3D map, Fig. 3. One can select this with the list box colour on the bottom right of the 3D map display. Choosing colour 'by intensity', switches one out of the default combinatorial chemistry colour 'by mass'. This display can partially compensate for the lack of adjacent background subtraction available in conventional mass spectrometry software e.g. smearing ion tracks that do not exactly parallel ions of interest can be eliminated from consideration.

The 3D map can be also thresholded according to % RA criteria i.e. only those peaks are displayed which have a % RA above the selected threshold. One can select a particular threshold with the list box 'Threshold' on the bottom left of the 3D map. On first switching to 3D map, the threshold is defaulted to 'dynamic' which rations displayable graphic points, well by well, rather than allowing noisy samples with many m/z values to grab a disproportionate share. If too much obscuring ion current exists then one can increase the threshold to eliminate it, which enhances the more important ion current e.g. 20% RA.

By clicking on a particular ion one can activate an information box displaying numerical data on that ion and well e.g., well number and actual m/z value. From this box one can return directly to the 3D map by mouse clicking an 'OK' button or to the respective spectrum by mouse clicking 'Show Spectrum'.

3.4. Chromatogram display

Since a histogram is the most obvious depiction for chromatograms or LC fraction sets, the three ion current schemes in the 'MS response' Overview i.e. base peak ion current, total ion current and total ion current multiplied by % purity were mapped to a chromatogram, Figs. 4–6, respectively. After choosing one of these three 'MS response' switches in Overview, these values are then instantly plotted versus well position on selecting the Chromatogram tab in the tab index. The underlying spectra are directly accessible through a mouse click on the respective well in the chromatogram display, Fig. 1. Two forms of data enhancement exist here. The total ion current, base peak ion current and total ion current multiplied by % purity, all reflect background subtracted spectra, providing a degree of spectrum cleanliness and baseline thresholding of the chromatogram. Secondly the base peak ion current depiction can provide a separate enhancement, especially in low analyte concentration/high background experiments. This is evident in the increased intensity of fraction F9 relative to its adjacent background fractions e.g., G1 to G4, in Fig. 4 compared with Fig. 5.

Since electrospray analyte ionisation efficiencies are usually solvent composition dependent, the intra-run relative intensity peak heights/areas in FIA–ESI-MS, with essentially constant solvent composition,

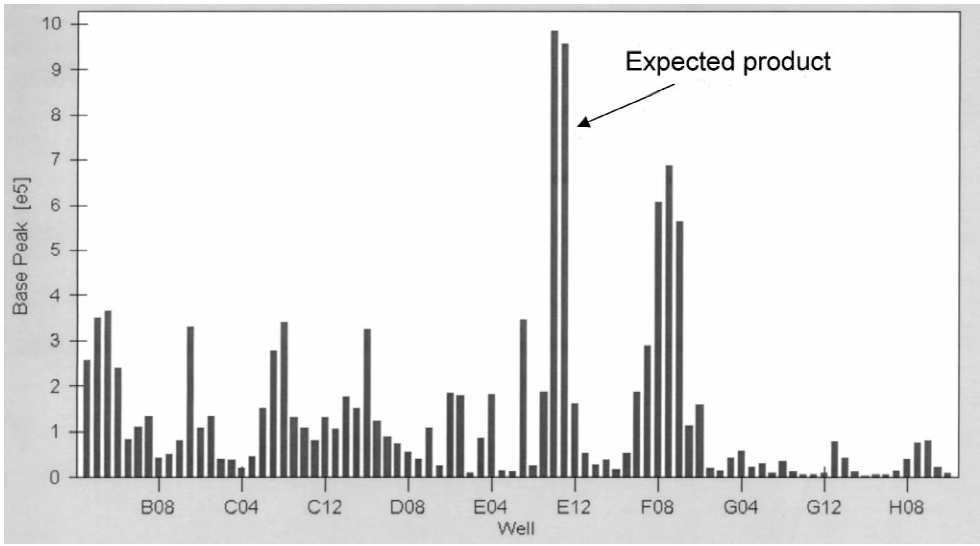

Fig. 4. Chromatogram of the positive ion ESI base peak ion current of each well. The underlying spectra are directly accessible. This base peak plotting provides enhancement when high backgrounds occur. To reach the colourisations in Figs. 4 and 5, one returns to Overview, picks the respective colourisation switch and replots 'Chromatogram'. The elution order is from left to right.

are more comparable than those from programmed organic solvent elution LC–MS.

3.5. Spectrum display

In the Spectrum display the MS spectrum of the selected sample is shown together with the results of the automated analysis. This includes total ion current and base peak ion current of the automatically background subtracted FIA–MS elution peak, Fig. 7. The spectrum which automatically takes its scan range from the mass spectrometer, is now a graph

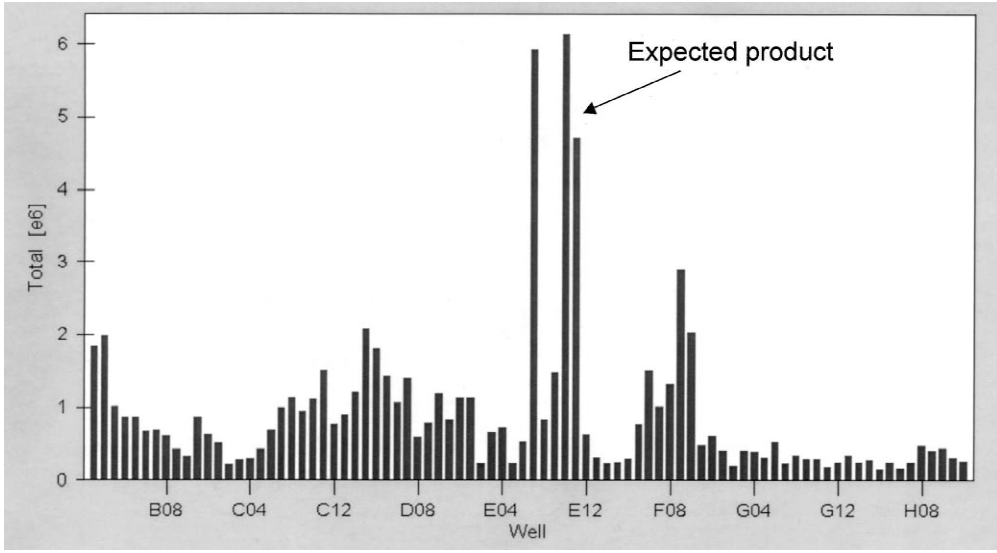

Fig. 5. Chromatogram of the positive ion ESI total ion current of each well. The underlying spectra are directly accessible. The elution order is from left to right.

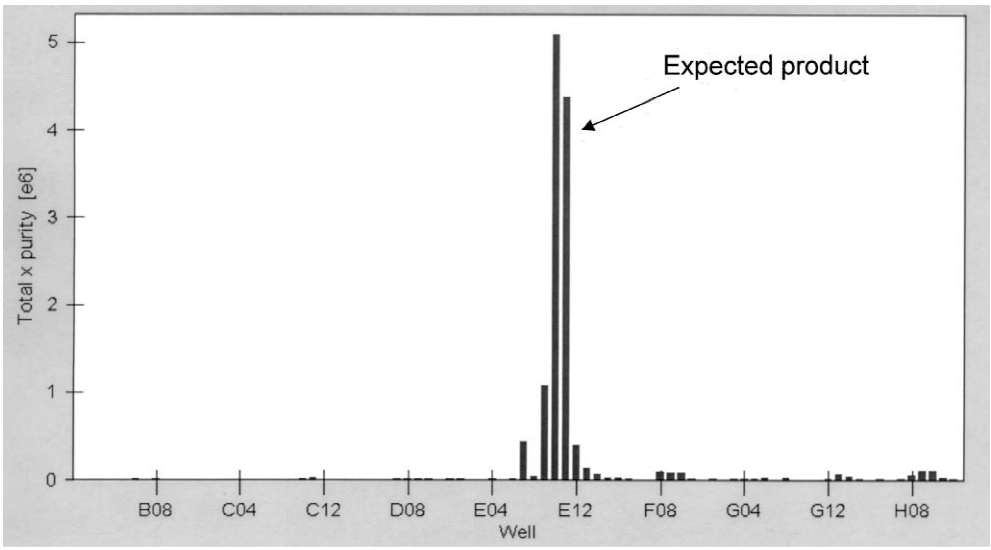

Fig. 6. Chromatogram of the positive ion ESI total ion current multiplied by % purity of each well. The underlying spectra are directly accessible. The elution order is from left to right.

which can be customised, printed and exported. In the top left corner of the spectrum the readout (m/z, % RA) of the current cursor position is numerically displayed.

3.6. Comparison of UV with MS data

All presented figures and data refer to the same LC run and consequent fraction set. A comparison of

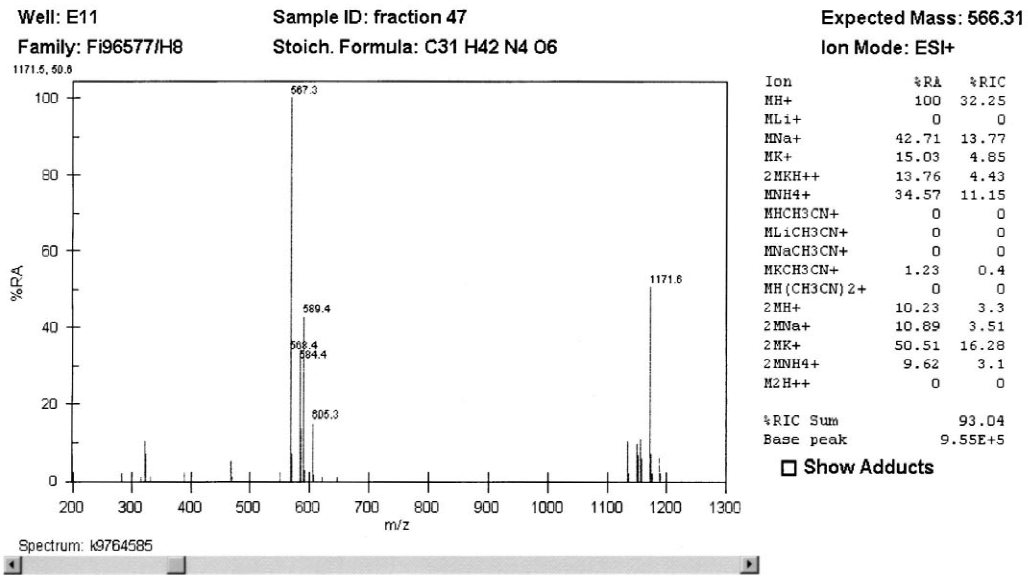

Fig. 7. Positive ion ESI spectrum of expected synthetic product with a monoisotopic molecular mass of 566.3, in well/fraction E11, showing the prominent $[M+H]^+$ and $[dimer+K]^+$ gas ions. The default mass scan range is relatively wide, to reach the gas dimers. The four gas dimers $[2M+H]^+$, $[2M+NH_4]^+$, $[2M+Na]^+$ and $[2M+K]^+$ were automatically incorporated into the purity estimation in Fig. 2.

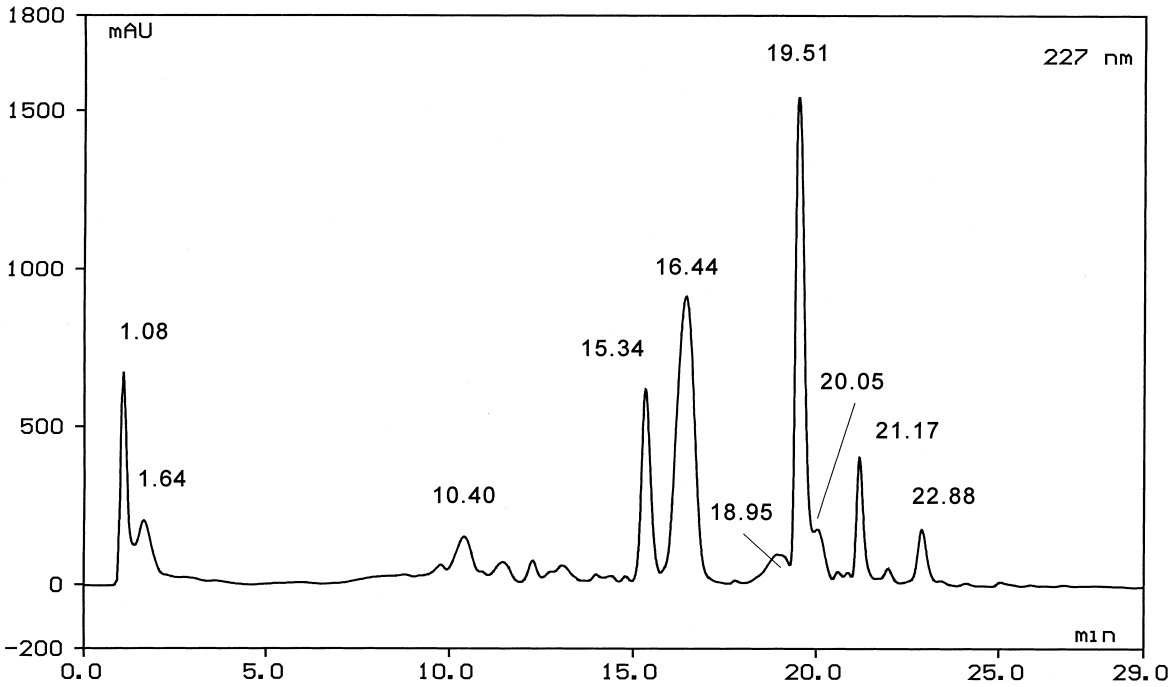

Fig. 8. LC chromatogram of tripeptoid analogue synthesis showing the three prominent UV peaks eluting at 15.34, 16.44 and 19.51 min (see Table 1). The expected synthetic compounds elutes at 16.44 min.

the UV chromatogram (Fig. 8) with the positive and negative ion ESI data (Table 1) emphasises the risk in using only LC–UV chromatograms at a single wavelength to scrutinise chemistry samples and the complementary nature of the three detection modes. Positive ion ESI is the default mode in the high-throughput FIA–MS. The D3 to D4, D11 to D12, F5 to F6 LC fractions with strong negative ion intensity

Table 1
Comparison of eluting compounds in three different detection modes i.e. at 227 nm (UV), negative and positive ion electrospray

LC fractions	UV retention time (min)	% Peak area at 227 nm	Monoisotopic molecular mass	
			Negative ion	Positive ion
C10 to C11		No peak	No peak	339
D3 to D4	10.40	3.5	462	462
D11 to D12	13.08	2.1	404	No peak
E7 to E8	15.34	8.7	263	263
E9 to E12	16.44[a]	24.5	566	566
F5 to F6	18.95	3.3	567	567
F8	19.51	21.4	No peak	580
F9 to F10	20.03	2.7	566	580
G1 to G2	21.17	4.4	250	No peak
G6 to G7	22.88	2.8	No peak	No peak

Two prominent peaks at 15.34 and 19.51 min could not be identified from their mass spectra as being directly related to the expected product[a].

and the C10 to C11, D3 to D4 and F5 to F6 fractions with strong positive ion intensity exhibit only minor or no peaks in the UV at 227 nm.

4. Conclusions

We report here the development of an automated high-throughput FIA–MS system for measuring LC fractions which includes the networked input of sample description by the synthetic chemists, and fast networked reporting of results to their work benches. An in-house Microsoft Visual Basic application called RACKVIEWER, allows easy and direct inspection of colour rendered spectral data by synthetic chemists from their workbenches, whether at the main corporate site or the various dispersed Novartis Pharma research institutes. Coloured computer screen pictures of individual well ion currents and 3D maps of the rack's entire ion current, provide a quick and easy way of delivering LC fraction data to laboratories in traditional synthetic chemistry, combinatorial chemistry and natural products chemistry. The spectra and resultant ion currents are automatically background subtracted and therefore provide intrinsic enhancement [4] for often noisy ESI spectra, simplifying interpretation by the non-specialist synthetic chemists. Other major advantages include a seamless integration of LC fractions into existing quick turnaround high-throughput MS queues while permitting the synthetic chemists a wider a priori choice (compared with conventional on-line LC–MS) of MS compatible chromatographic conditions. Lastly the high-throughput FIA–MS allows the unattended MS screening of large numbers of contiguous LC fractions. This reduces the risk of missing compounds with low UV absorbance, by replacing the conventional practice of synthetic chemists in designating, based on UV response, only a limited number of semipreparative and analytical LC fractions, to be sent to MS laboratories.

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References

[1] B.E. Bauer, in: I.M. Chaiken, K.D. Janda (Eds.), Molecular Diversity and Combinatorial Chemistry: Libraries and Drug Discovery, American Chemical Society, 1996, pp. 233–243.
[2] G. Hegy, E. Görlach, R. Richmond, F. Bitsch, Rapid Commun. Mass Spectrom. 10 (1996) 1894–1900.
[3] E. Görlach, R. Richmond, I. Lewis, Anal. Chem. 70 (1998) 3227–3234.
[4] W. Windig, J.M. Phalp, A.W. Payne, Anal. Chem. 68 (1996) 3602–3603.
[5] H.E. Hail, B.M. Warrack, A.S. Arroya, G.C. DiDonata, M.S. Lee, Proceedings of the 44th ASMS Conference on Mass Spectrometry and Allied Topics, Portland, 12–16 May, 1996, p. 1038.
[6] S.S. Smart, T.J. Mason, P.S. Bennell, N.J. Maeji, H.M. Geysen, Int. J. Peptide Protein Res. 47 (1996) 47–55.
[7] F.S. Pullen, D.S. Richards, Rapid Commun. Mass Spectrom. 9 (1995) 188–190.
[8] L.C.E. Taylor, R.L. Johnson, R.J. Raso, Am. Soc. Mass Spectrom. 6 (1995) 387–393.
[9] D.M. Garcia, S.K. Huang, W.F. Stansbury, J. Am. Soc. Mass Spectrom. 7 (1996) 59–65.
[10] F.S. Pullen, G.L. Perkins, K.I. Burton, R.S. Ware, M.S. Teague, J.P. Kiplinger, J. Am. Soc. Mass Spectrom. 6 (1995) 394–399.
[11] R.N. Zuckermann, J.M. Kerr, S.B.H. Kent, W.H. Moos, J. Am. Chem. Soc. 114 (1992) 10646–10647.
[12] J.L Whitney, M.E. Hail, R.A. Rourick, K.J. Volk, E.H. Kerns, M.S. Lee, Proceedings of the 44th ASMS Conference on Mass Spectrometry and Allied Topics, Portland, 12–16 May, 1996, p. 1046.