

# Chromatography

## Visiting the 15th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology (HTC-15)

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The 15th International Symposium on Hyphenated Techniques in Chromatography was held in Cardiff, Wales from January 24th to January 26th 2018. An optional series of short courses on 'Introduction To Biopharmaceutical Analysis', 'Supercritical Fluid Chromatography: From Theory To (Industrial) Application', and 'The Statistical analysis of chromatographic data: A practical guide'; were held the day before HTC-15 on Tuesday 23rd January 2018.

The location for HTC-15, was Cardiff, the capital and largest city in Wales and the eleventh-largest city in the United Kingdom. Cardiff was a small town until the early 19th century when its prominence as a major port for the transport of coal following the arrival of industry in the region contributed to its rise as a major city. Cardiff was made a city in 1905 and proclaimed the capital of Wales in 1955.

The symposia were again jointly organised by the Royal Flemish Chemical Society (KVCV) and the Separation Science Group of the Royal Society of Chemistry (RSC) and took place in the Cardiff City Hall.

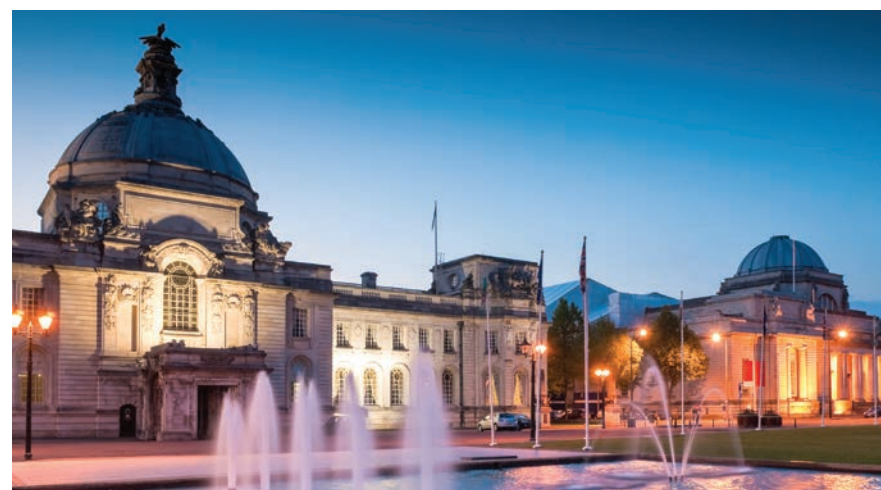
The magnificent Edwardian Cardiff City Hall is actually the fifth to have served as the centre of local Government. Little is known of Cardiff's original 'Gild Hall', but the second Town Hall stood in the middle of St Mary's Street until it was replaced on the same site in the mid- eighteenth century. The fourth Town Hall (before Cardiff became a city), on the western side of St Mary's Street, was built in 1853 and remained in use until the present City Hall, dominated by the 194-foot-high clock tower, opened in 1904.

The site was very grand and ornate, as with many historic city hall sites; the meeting rooms housed three parallel sessions easily, and the Assembly hall, where the Wednesday mixer, coffee breaks and lunches were presented, provided ample space for the posters and exhibition area housing a total of 64 poster and 25 exhibitors (an increase from the 15 exhibitors at HTC-14).

As usual, industry (40%), academia and students (60%) were all well represented among the speakers and over 290 delegates from 21 different countries. Early career researchers, an important focus of HTC, provided nearly 30% of the total presentations

When asked what the expectations for HTC-15 were, John Langley the HTC and RSC Separation Science Group Chairman said: "the expectations of the RSC Separation Science Group for HTC 15, was to build on the ethos of previous meetings, provide an exciting scientific program with academic and industrial speakers, host an associated exhibition and particularly make space for presentations from early career researchers, who are the future of our scientific field." He continued: "This was achieved through working with an excellent committee that included KVCV, the Chromatographic Society and the British Mass Spectrometry Society. Particular mention must go to Tom Lynch and Ruth Godfrey who shouldered the majority of the organisation with me."

HTC-15 covered many innovative applications based on hyphenated techniques and a large section of the meeting considered the production and consequences of 'big data' produced in current hyphenated analytical techniques, taking place in 3 concurrent parallel sessions, making it difficult and a little frustrating when attempting to participate in the multitude of interesting discussions. The flash Poster session started at HTC-13 was continued with great success; three scientific vendor lunchtime seminars (Leco, Sciex and Ellutia Ltd) were also available and a recruitment lunch seminar was presented aimed at 'Securing your perfect job'.



This review will now focus on a selection of the presentations given during the HTC-15 meeting. However, an overview of the main topics covered during the symposium is given here:

- Exploiting Separation Science
- (R)evolutions in Biopharmaceutical Analysis
- Fundamentals in Separation Science
- Advances in Clinical Analysis
- Interfacing and Ionisation
- High throughput versus High Efficiency Separations
- Exploiting Separation Science and Mass Spectrometry
- Microfluidics & Flow Process
- Big Data - What do we do with it?
- Separations by shape: Instrumentation and Further applications of chromatography
- Green Separations
- Big Data Chemometrics and Method development (In-Silico)
- Approaches to maximising analytical data
- Ion Mobility - Mass Spectrometry
- Environment Pollutants, what can the data tell us?
- Automating Complex Sample Workflows
- Comprehensive Chromatography - The State of the art
- Energy & the Environment
- Analysis of Complex Energy Products
- Advanced Analysis of Food and Beverages
- Life science & Pharma

The opening of HTC-15 was chaired by Dr John Langley (University of Southampton, UK) and preceded the award of the Knox Medal. The Knox Medal is presented to 'honour individuals deserving special recognition of their innovation or influential work in the field of Separation Science', the HTC-15 Knox Medal award was presented by John Langley (RSC) to Professor Peter Myers (University of Liverpool, UK) who captivated the packed auditorium with the plenary lecture entitled 'Why Are We Still Using Silica?'



Peter after thanking the many people that have helped him reach this point in his career, entertained the delegates to an entertaining and challenging lecture being very passionate, as he always has been, about the use of silica as an HPLC adsorbent. He bestowed the benefits of silica (rigid, robust and easy to make) and then highlighted the problems of silica (dissolution in water, particle size distribution issues, surface silanols and acidity, bonding leaching at pH's below 2 and pore size measurement issues). The work of Knox and Kaur; European Chromatography Review 1987 in detailing an ideal HPLC adsorbent material template was then described and how that led to the Knox development of Hypercarb (a graphitic carbon material manufactured from a silica template).

Peter then moved on to discuss pillar arrays, his work on electrophoresis with Dr Keith Bartle, dynamic field gradient focusing, centrifugal chromatography using a CD, ultra-porous metal organic framework materials and his team's most recent work, at the University of Liverpool, on the use of field effect transistors made from graphite.



Professor Peter Myers receiving the Knox Medal from Dr John Langley

The three parallel sessions started after the morning coffee break with the '(R)evolutions in Biopharmaceutical Analysis' session chaired by Deirdre Cabooter being well attended. Oral presentations were given on:

- The Keynote: Recent advances in the analysis of protein biopharmaceuticals. Koen Sandra, Research Institute outlined the versatility of hydrophilic interaction chromatography (HILIC), the benefits of instrument and column inertness, the power 2DLC and the possibilities of micropillar array columns ( $\mu$ PAC), all hyphenated to high-resolution mass spectrometry (MS). The use of alternative procainamide labels to enable increased detection limits was also outlined
- Prototyping of a microfluidic multilayer modulator chip for multi-dimensional separations. Jelle De Vos, Vrije Universiteit, Brussels (this presentation replaced the one scheduled by Davy Guillaume, University of Geneva, who sadly could not attend the meeting). Jelle presented an optimised trap configuration for refocusing and elution/remobilisation conditions showing signal enhancement factors in the order of 10 to 20 being achieved
- Advancing the analytical toolbox using shotgun lipidomics for lipid modifying enzymes. Dr Erwin Kaal, DSM discussed how the present workflow for lipid analysis is time consuming, gives relatively large analytical errors and some details on the lipids cannot be obtained and showed a newly developed ion mobility-based shotgun lipidomics approach.
- Lipidomics by UHPLC-QTOF-MS/MS with Data-Independent Acquisition and Clinical Applications. Michael Laemmerhofer, University of Tuebingen presented several applications of the use of data independent acquisition workflows employing SWATH technology and showing how in platelet lipidomics using MS-DIAL deconvolution >1100 ESI +ve and 300 ESI -ve molecular features.

The 14:00-15:30 afternoon session selected for review was on 'Fundamentals in Separation Science' chaired by Dr Sebastiaan Eeltink, featured four oral presentations on:

- Recent Progress in the Development of Perfectly Ordered Separation Media. Gert Desmet, Vrije Universiteit, Brussels illustrated and demonstrated how micro-machining technology can boost High Performance Liquid Chromatography (HPLC) using advanced photolithographic etching techniques such as the Bosch-process to produce perfectly ordered porous support columns with optimised hydrodynamic shape and optimised external porosity.
- Considerations for the use of ultra-high pressures in liquid chromatography for 2.1mm inner diameter columns. Ken Broeckhoven, Vrije Universiteit Brussels gave an overview of the effects of viscous heat dissipation in chromatographic columns (with an emphasis 2.1mm columns) using numerical simulations of the temperature and velocity profiles and the resulting band broadening, for the first time at operating pressures up to 2000 bar, i.e. the expected operating pressure of LC instruments of the future.
- Matching 1st and 2nd dimension chemistries in the 2D-LC. Active Solvent Modulation. Konstantin Shoykhet, Agilent Technologies discussed the different optimisation strategies and the factors limiting speed in multidimensional analysis.
- Kinetics and mass transfer phenomena in modern chiral stationary phases, Martina Catani (replaced Alberto Cavazzini – who could not attend), University of Ferrara, discussed the state-of-art techniques that have been employed to study mass transfer in modern chiral stationary phases (CSPs) providing results that disagree with the supposed superiority of chiral superficially porous particles (SPPs) over their fully porous counterparts to achieve high efficient, ultrafast enantioseparations.

A brief 30-minute coffee break permitting attendees of the conference to view the posters and exhibits was followed by a second afternoon session from 15:30-17:30. The session reviewed was 'Microfluidics & Flow Process Technology' which covered:

- Flow chemistry: A synthetic chemist's perspective. Anna Slater, University of Liverpool. Anna started by confessing she was not an analytical chemist and 'like her 3-year old – she may come to us and say how can I clean up this mess'. She proceeded to explain batch and flow reaction chemistries and the need for the hyphenation of analytical tools to provide key reaction state information.

- Chemically Sensitive Online Detectors for SEC – current advances for SEC-MR-NMR and SEC-QCL-IR. Johannes Höpfner, Karlsruhe Institute of Technology (KIT). Method development for SEC-QCL-IR and MR-NMR-SEC including the general setup, the sensitivity enhancement, the solvent suppression and the influence of chromatographic conditions were presented.

- Comprehensive Two-Dimensional Liquid Chromatography Coupled to Triple-Detection for Characterization of Branched Polymers. Nico Apel, Fraunhofer LBF showed how a new chromatographic method, namely solvent gradient at near-critical conditions, was developed by applying a mobile phase gradient in a very narrow range around the critical point that permitted the separation of polymers according to the end-group moiety of the analysed polymer.

- Chromatographic strategies combining RPLC, mixed-mode HPLC and SFC coupled to MS for impurity profiling of drugs candidates. Elise Lemasson, Institut de Chimie Organique et Analytique (ICOA) discussed the development of alternative analytical methods to reversed-phase HPLC on C18 phase for impurity profiling of 140 pharmaceutical compounds. Reversed-phase HPLC on other stationary phases (e.g. penta fluorophenyl phases), mixed mode HPLC (combining reversed-phase and ion-exchange mechanisms) as well as SFC were shown.

After the final presentation on Wednesday evening a Mixer (sponsored by Leco) was held in conjunction with the exhibition and poster viewings. The area was well laid out and made for a great atmosphere where everyone mingled, sampled the beers and enjoyed passed hors d'oeuvres, all whilst talking hyphenated chromatography.



The Wednesday Mixer

An extremely well attended ticket only Beer Degustation Social with food followed at 19:30 located in the Brewery Quarter at The Yard, St Mary Street, Cardiff.

On Thursday morning a professional development and careers breakfast workshop starting at 08:00 was followed by the HTC announcements and then a very stimulating and thought provoking plenary lecture at 09:00: Transforming Big Data into Big Analysis: The Power of Finding Use-Value In Your Data by Eric Little, OSTHUS. Eric showed how by 2020 there will be 2.3 Zetabytes of internet data traffic annually and explained the 4 V's of big data (Volume, Velocity, Variety and Veracity) and how these are treated by analytics, statistics and semantics approaches and how these related to the analytical measurements produced.

The subsequent selection of one of the three parallel sessions before the morning coffee break, 'Separation Science Group session' was presented in a keynote, two lectures and an award presentation including:

- Advancing hydrophobic interaction chromatography methods to characterise biotechnology enzyme mixtures and to profile biotherapeutics. Sebastiaan Eeltink, Vrije Universiteit, Brussels discussed a systematic investigation to assess retention properties and selectivity of a hydrophobic interaction chromatography (HIC) stationary phase. The adsorption process by salting out was discussed highlighting the hydrophobic patches on the protein surface which interact with the hydrophobic stationary phase. Issues with HIC were identified and a mechanism for separation proposed.
- Methodologies to determine b-term coefficients revisited. Deirdre Cabooter, University in Leuven presented three commonly used methodologies (peak parking; fitting of an experimental plate height curve; and the so-called dynamic method) for the determination of the b-term constant.
- UHPLC quantitation and identity confirmation in drug development with a multi-detector approach. Frank Steiner, Thermo Fisher Scientific discussed an approach that relies on a multidetector set-up based on LC-UV-CAD-MS. The single quad MS provides m/z values of the peaks to ensure the correct peak assignments and enables the detection of co-eluting compounds. The CAD provides universal quantitation, based on a calibration curve. The UV detector complements the data collected by MS and CAD and acts as valuable tool for method troubleshooting and method transfer.
- The inaugural LCGC Europe/HTC innovation award 2018 was awarded to Professor Carolin Huhn, University of Tubingen for her work on multidimensional separations in the electric field. The award was launched to celebrate innovative achievements in hyphenated techniques that benefit society and was selected by a judging panel from the HTC15 organising committee





Professor Carolin Huhn with The inaugural LCGC Europe/HTC innovation award 2018

The post lunch session 14:00 covered, 'Green Separations', with a keynote and three oral presentations:

- 50 shades of Green SFC. Caroline West, University of Orleans discussed the question of 'how green is SFC' and the features that should define green chromatography such as the generation of waste and the use of toxic chemicals, energy consumption, extra chemical reactions (derivatisation) for solubility, separation or detection, possible choices of less toxic co-solvents, analysis time, method development time, and the risk of accidents. A fair examination yielded that reverse phase UHPLC is as green as SFC.
- Can CE-MS improve the detection of peptides and intact proteins and in biological samples? Stephen Lock, Sciex described how a CESI-MS method was developed to detect intact neuropeptides of the size of PACAP (Molecular Weight of 4534.26 amu) and VIP in biological samples overcoming some of the analysis challenges presented by LC-MS.
- UHPSFC-MS of a Range of Steroidal Compounds. Julie Herniman, University of Southampton presented applications of UHPSFC-MS of a range different steroidal compound classes and their introduction into an open access environment to facilitate the analysis of archaeological materials, biofuels and to monitor chemical synthesis.
- Online extraction and determination of carotenoids from food sample by means of supercritical fluid extraction-supercritical fluid chromatography-mass spectrometry. Mariosimone Zoccali, University of Messina discussed the development of an online method coupling supercritical fluid extraction and supercritical fluid chromatography (SFC) for the extraction and detection of carotenoids from Tamarillo samples and the comparison to offline methods, showing the capability of the online method to greatly reduce the analytical times and solvent consumption.

The Thursday afternoon 16:00 to 17:30 parallel sessions, covered Screening Environment Pollutants, what can the data tell us?

- Urban water profiling to inform the state of the environment and public health. Barbara Kasprzyk-Hordern, University of Bath introduced the concept of urban water fingerprinting with hyphenated mass spectrometry techniques and its rapid advances particularly focused on pharmacologically active compounds in urban water and their stereochemistry in the context of environmental risk assessment.
- Suspect screening of aquatic environmental matrices using high resolution analysis and in silico tools for broad scope tentative contaminant identification. Leon Barron, King's College London explained how a selection of analytical approaches had been developed for suspect screening of river water and wastewater in London for compounds of both forensic and environmental interest such as drugs of abuse, contraceptive hormones and TNT.
- Screening of environmental passive sampling extracts using LC Q-TOF-MS in data-independent acquisition mode. Anthony Gravell, Natural Resources Wales elucidated on how an alternative methodology based on data-independent MS/MS overcame the disadvantages of the other two approaches targeted MS/MS and data-dependent MS/MS.
- Combining high-capacity sorptive extraction with Thermal desorption pre-concentration for analysis of (S)VOCs in environmental samples. Lara Kelly, Markes International Ltd discussed the benefits of using thermal desorption (TD) sample introduction and how it extends to the ability to re-collect a sample for repeat analysis, assisting with method validation and eliminating the need to perform repeat extractions on limited sample quantities.

The HTC-15 Conference Gala Dinner the main social event of the symposium was held on Thursday Evening (19:15) in the National Museum Cardiff, where a floor of the art exhibition was opened to the attendees who could, with a glass of prosecco in hand, peruse the art on display. After approximately 30 minutes we were all called to proceed for dinner and were welcomed by the 55 strong Blaenavon Male Voice Choir. Ranging in age from teenagers to people in their eighties, and drawn from towns around Blaenavon, the choir is primarily occupied in performing concerts for charity purposes and was obviously warming up for their singing of the national anthems at the Wales v Scotland Rugby International match on 3rd February, 2018.



The Blaenavon Male Voice Choir

The Friday opening plenary lecture by Tuulia Hyotylainen on 'Hyphenated Techniques for comprehensive analysis of all metabolites in biological systems to describe metabolic changes caused by disease, environmental, nutritional, or genetic factors' was followed by the session selected for review was on 'Comprehensive Chromatography - The State of the art' chaired by Hans Gerd Janssen and featured four oral presentations. The oral presentations were as follows:

- Flow Modulated Two-dimensional Gas Chromatography Coupled to Tandem Mass Spectrometry for 'Comprehensive' characterisation of Complex Samples. Luigi Mondello, University of Messina – discussed the advantages of using a flow-modulation device in GCxGC when compared to a cryogenic one, showing compatibility with virtually any detector and flow rates with reduced costs and environmental risks. A series of complex flow-modulated GCxGC-MS real-world sample applications were shown.
- Applicability of retention modelling in hydrophilic-interaction liquid chromatography for algorithmic optimisation programs with gradient-scanning techniques. Bob Pirok, University of Amsterdam showed how to facilitate rapid method development of LCxLC methods, using the newly developed Program for Interpretive Optimization of Two-dimensional Resolution (PIOTR), allowing rigorous, comprehensive optimisation of LCxLC methods by interpreting a very limited number of scouting LCxLC(-MS) experiments and hence reducing complex method development time.
- All Ion Differential Analysis in Product Control Applications using GC/MS and Comprehensive GCxGC/MS. Marco Ruijken, MsMetrix discussed that while GCxGC-MS has become an invaluable laboratory analysis tool, the procedure produces gigabytes of data per sample in four dimensions, making data analysis time consuming and complicated. New methods and software tools applicable to GC/MS and GCxGC/MS were presented finding differential components from a comparison of two samples.
- Detection of bacteriologically produced hydrogen sulphide using SHS-MCC-GC-IMS. John Dean, Northumbria University discussed a new method that offers great promise for a rapid and extremely sensitive analytical technique for the detection and quantification of H<sub>2</sub>S commonly associated with bacterial contamination of food and water sources.

The final session of lectures on Friday afternoon – 'Life Science & Pharma' commenced with a rapid-fire tutorial and three oral presentations:

- LC-MS small molecule quantitation: a short tutorial of best practice. Ruth Godfrey, Swansea University presented a tutorial inspired by guidance prepared on behalf of Analytical Methods Committee (AMC) of the Royal Society of Chemistry (RSC) which was designed to help inform and support the use of LC-MS for small molecule quantitation.

- From GC-MS to LC-MS/MS: Further Advances in Adrenal Cancer Diagnosis, Angela Taylor, University of Birmingham outlined the challenges in discriminating adrenocortical adenoma (ACA) from adrenocortical carcinoma (ACC) in patients with adrenal masses and the development of a novel LC-MS/MS method producing a significant advance diagnosis of ACC.
- Green Bioanalytical Analysis of Voriconazole and Tadalafil by HPLC. Aysegul Dogan, Hacettepe University showed how 30 drug molecules having different physicochemical properties were analysed firstly with conventional organic modifier based HPLC and then with propylene carbonate modified mobile phases and how three of the molecules with the best chromatographic separation were selected for analysis from plasma.
- Improving Untargeted Metabolomics with Ion Chromatography-Mass Spectrometry. John Walsby-Tickle, University of Oxford discussed the development of an IC-MS technique for targeted and untargeted applications and demonstrated exceptional retention time stability and lower limits of detection when compared to HILIC.

The closing ceremony and farewell event took place in the lower hall on Friday afternoon

- Flash Poster Presentations
- Plenary by Rob Beynon - Of mice, sex and mass spectrometry (Chair: John Langley)
- Plenary by Peter Schoenmakers - Multi-dimensional liquid chromatography of complex mixtures (Chair: Frederic Lynen)
- Poster Awards
- Farewell Event and handover of the meeting to HTC-16 Ghent.

Poster prizes were awarded for the posters which showed originality and novelty of the work performed and outstanding visual presentation, the first and second of which were awarded to Mathijs Baert - £250 cash prize, (Ghent University) and Kris Wolfs - £150 cash prize respectively, (Pharmaceutical Analysis - KU Leuven) for their posters titled – ‘Exploring the possibilities of temperature responsive columns in comprehensive two-dimensional liquid chromatography’ and – ‘Application of an Atmospheric Micro Hollow Cathode Discharge Set-up in a Novel GC-detector’ respectively. The third prize was awarded to Vincent Pepermans - £100 book prize, (Vrije Universiteit Brussels (VUB)) for the poster titled – ‘Enhancing detection limits in chromatography using solvent-assisted post-column refocusing’.

After this very successful 15th edition of the symposium on Hyphenated Techniques in Chromatography the next and 16th edition will be organised in Ghent, Belgium, during the last week of January 2020.

When asked for his thoughts on the success of HTC-15, Dr Langley answered: “The meeting exceeded all expectations, in scientific content, lecture quality, networking and interaction with our exhibitors, and the social program that followed the norm but with a very Welsh theme. This meeting could not have been delivered without the support of the fantastic professional congress organisers, particular thanks must go to David Hellyer and Lucinda Johnstone of ILM Exhibitions.”

When asked what was next for HTC-16 in 2020, he replied: “The baton has now been returned to our Belgian colleagues and KVCV for HTC 16 in Ghent 2020 whom I am confident will build on the success of Cardiff meeting and continue the HTC series as the premier event for hyphenation.”