Journal of Chromatography B, 879 (2011) 1157–1158

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Preface

Enhancement of analysis by analytical derivatization

Understanding the fundamental principles of actions and interactions between molecules and the application of such understanding to human needs is the role and intellectual pleasure of the chemist. The premise of this Special Issue is that application of chemistry to analytical derivatizations during sample preparation can greatly improve both the quantitative and qualitative results of analysis.

Based on reviews and original articles herein, this premise is well justified. Increase in sensitivity subsequent to derivatization is well established for gas chromatography (GC) and high-performance liquid chromatography (HPLC). Silylation increases the volatility and often stability of the analytes. The resulting improvement in chromatography delivers more analyte into the detector in a narrower chromatographic peak thus increasing sensitivity. Adding a chromophore or fluorophore to an analyte devoid of such properties greatly decreases the limits of detection or quantitation. These effects are not surprising. Increase in sensitivity via derivatization, however, also occurs for mass spectrometric (MS) detection which is the gold standard for analytical methods. This observation holds even as the sophistication and capability of mass spectrometric instrumentation evolved from GC–MS, to LC–MS with various ionization techniques as well as MALDI and FTICR.

Derivatization is applicable to any functional group, to numerous classes of analytes and to a wide range of instrumentation. It serves at one time to enhance sensitivity and as a functional group analysis which enhances specificity. The 2,3,4,5,6-pentafluorotoluene class of reagents are one example. The five fluorines on the aromatic ring impart the electrophoric properties for enhanced detection by GC with electron capture detection, GC–MS with negative ion chemical ionization and are also effective for improving sensitivity of LC–MS. Modification of the methyl group with various functionalities determines the reactivity. Thus pentafluorobenzyl bromide will react with nucleophiles including ionized organic acids, amines and inorganic anions. Pentafluorobenzoyl chloride reacts with alcohols and amines, and pentafluorbenzylhydroxylamine derivatizes carbonyls. Similarly, reagents with the DANSYL fluorophore, which imparts fluorescence through the naphthalene moiety, are suited for high sensitivity HPLC analysis and are also applicable to many functional groups. DANSYL-Cl forms derivatives with hydroxyl and amino groups whereas DANSYL hydrazine reacts with the carbonyls. By virtue of the tertiary amine in DANSYL reagents their derivatives are ionized at acidic pH and enter the ion source of the mass spectrometer as a cation and do not require ionization. Increase in sensitivity can be as high as 3000 fold. The Girard reagents which possess a quaternary amine also introduce a pre-charged species into the ion source and likewise substantially improve the sensitivity of mass spectrometric detection. First reported in 1936 this class of carbonyl specific reagents were used to separate keto and phenolic steroids isolated from urine or tissue. In their most recent incarnation, the Girard (and/or Girard-like) reagents introduce a permanently pre-charged species for mass spectrometric detections and are thus applicable to separations at all pH values.

Improvement in sensitivity, while important, is only one function of analytical derivatization. Products formed in these reactions are usually more lipophilic than the analyte and are thus easier to extract and concentrate in the final isolate. This is particularly important for small molecules which are invariably hydrophilic. In the determination of low-molecular weight compounds, derivatization proves to be an important front end procedure for detection even for high-end mass spectrometric detection.

The flexibility of analytical derivatizations makes this technique transferable to between matrices and applications. Aldehydes and ketones are present in both breath and atmospheric air and require the same analytical derivatizations to isolate these low-molecular weight compounds. Measurement of amino acids is integral for the study of numerous biological systems ranging from wine – which is a microbiological system – to disease states in humans. And again the same chemistry applies.

A major challenge in analytical derivatizations is often posed by excess of reagent. Standard separation techniques are useful in some instances. Pentafluorobenzyl bromide has a lower boiling point than many of the derivatives and in these cases selective evaporation is feasible. Clearly this would not be the case for DANSYL or Girard/Girard-like reagents. Semi-preparative chromatography is an option but it adds to the complexity of a method. In other instances, injection of the isolate onto the analytical column and superior GC or HPLC can separate reagent from derivatized analyte. The latter option, however, often requires dilution of the sample in the injection solution and less than optimum sensitivity.

A powerful technique for resolving the problem of excess reagent is preparation of reagents that have different physicochemical properties than the derivatives of the analyte. For instance, OPA/thiol reagents are non-fluorescent but they form derivatives with primary amines that exhibit very high fluorescence while leaving the reagents invisible to the detector. Reagents that have a highly fluorinated alkyl leaving groups at the reactive functionality, react with amines which displace that leaving group. The result is a derivative with a detection moiety (e.g. a fluorophore) while the reagent retains the fluorinated moiety. The reagent is then separated from the derivative using a SPE column packed with

1570-0232/$ – see front matter
doi:10.1016/j.jchromb.2011.04.010

a highly fluorinated reverse phase material which strongly binds fluorinated derivatives.

Given both the advantages and disadvantages of analytical derivatization it behoves chemists to develop techniques, methods, and procedures that exploit the former and diminish the latter. There is an abundance of evidence that this can be done. Programmable auto-injectors can mix reagents and analyte and so automate many analytical derivatizations. Development of solid-phase techniques for analytical derivatization is an important step in this direction. It permits use of the well-established SPE or SPME devices and instrumentation to simplify and automate sample preparations that involve derivatizations. Although liquid–liquid extraction is the first technique for isolating analytes from aqueous solutions it remains a vibrant field. Liquid-phase micro-extraction whether as single drop, hollow-fibre or dispersed phase reduces volumes of solvent and amounts of reagent required as well as increases throughput with benefit to both environment and cost of analysis. As in the case of solid-phase extractions, investigators in liquid-phase extractions incorporate analytical derivatizations into the extraction techniques to take advantage of the instrumentation developed for isolation. In the modern world speed of processes becomes a much desired quality. To this end ultrasonic and microwave irradiation enhance reaction rates and throughput. Fast reaction times can reduce exposure of labile samples to air and light.

The applications of organic analytical chemistry are most widely seen in the client sciences such as medicine or environmental monitoring. In these client sciences there is a constant drive towards faster, more sensitive and less expensive techniques. The reports in this Special Issue show that analytical derivatizations have the potential to meet these needs. They challenge analytical chemists to devise techniques and instrumentation – particularly those that are highly automated – that take advantage of the increases in sensitivity and specificity but also reduce or eliminate the disadvantages of derivatizations. As always, the rising to challenge will address and meet these needs but it will also produce new ideas and interesting chemistry.

I would like to thank the authors for their contributions to the Special Issue on Analytical Derivatization which will inform colleagues about the advantages, challenges, and application of these techniques. And I extend my thanks to Elsevier, the Editors and staff of the Journal and particularly to Dimitrios Tsikas for their guidance and encouragement in the preparation of this issue.

Jack Rosenfeld is a Professor Emeritus in the Department of Pathology and Molecular Medicine at McMaster University. His work derived from the needs of analysis in biomedical research. Those needs of high sensitivity and specificity, ease of analysis and wide application guided his study into analytical derivatizations in general and the development of solid phase analytical derivatizations in particular. The focus of studies on SPAD has been to establish the basic chemistry and apply this knowledge to automating the methods. Currently he is working on extending these reactions to the development of simplified techniques for determining the broad range of analytes encountered in environmental monitoring. Here the focus is on reducing the sample size, volumes of solvents used in isolation and finally automation. He has published original articles, reviews, book chapters on these topics and edited a book on sample preparation for hyphenated techniques. Although educated as a chemist, he has also served as a tutor, administrator and investigator in medical education. He has been instrumental in development of evaluation and admissions techniques at the Michael G. DeGroote School of Medicine at McMaster. One of these recent developments – the Multiple Mini Interview – is now used for selection of students to medical and professional schools in Canada and in other countries around the world.

Jack Rosenfeld *
Department of Pathology, McMaster University, Hamilton, ON, Canada

* Tel.: +1 905 628 8867; fax: +1 905 521 2613.
E-mail address: rosenfel@mcmaster.ca

Available online 14 April 2011