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Letter to the Editor

Satellite high-performance liquid chromatography for trace sample cleanup

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The general problems of losses, interferences and contamination associated with sample cleanup are familiar to those who perform trace analysis. Sorting these problems out in a multistep method, especially when they are irreproducible and the amount of the analyte is barely detectable in the first place, can be a tedious undertaking. In part this explains why there are many more publications in which the trace measurement of pure standards rather than real samples are reported.

Two techniques that are often considered for trace sample cleanup are solid-phase extraction (SPE) and HPLC. SPE can be convenient and minimize contamination since disposable devices can be employed. However, the inherent separation power of SPE tends to be limited, and reproducibility can be a problem when different batches of SPE packings are tested, even from the same manufacturer. HPLC can provide a reproducible, high-resolution separation, but the usual system is relatively expensive and typically is applied to several projects in a laboratory. Thus, at the trace level, the system tends to be dirty and thereby can substitute, irreproducibly, one set of interferences for another when applied to a trace sample. Such a system also can contaminate a trace sample due to carryover of analyte derived from the prior injection of a calibrating sample [1].

Satellite HPLC is a partial remedy for these types of difficulties. We suggest this terminology for an isocratic HPLC system that consists of just a solvent reservoir, low-cost but high-precision pump, injector and column. Such a system can be set up for less than about US$ 2500, and one or more such systems can be operated in conjunction with a more ordinary parent HPLC which has gradient capability and incorporates a detector. The equipment that we have used to set up satellite HPLC in our laboratory is as follows: model CC-6-S pump from Eldex Laboratories (Napa, CA, USA), and model 7725 injector from Rheodyne (Cotati, CA, USA). We have not tested other injectors for this purpose. A device to control the column temperature should be included for some applications.

For method development, one first tests the column of interest on a parent HPLC, where a detectable amount of standard analyte (e.g. ng–μg for on-line UV detection) is measured to develop the separation conditions. The column is then transferred to the satellite HPLC for cleanup of trace samples. Contamination of the satellite HPLC is avoided since the usual cause of this is the injector [1], which stays with the parent HPLC. As we summarized before [1], the sample is exposed to a variety of potentially adsorbing surfaces, along with crevices, in this injector. By dedicating the satellite HPLC to the trace level, and even to a particular purification step

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in a method, contamination due to sample carryover, as well as interferences, can be more easily controlled. Extra care is essential in preparing the mobile phase and controlling or monitoring the temperature of separation on the satellite HPLC to establish a known retention time for the analyte, especially when off-line detection is done. As necessary, the column can be returned periodically to the parent HPLC in case its retention properties need to be retested.

We were motivated to employ satellite HPLC when we observed the enormous cleanup that HPLC could provide of a sample prior to off-line detection by gas chromatography–electron capture mass spectrometry (GC–EC-MS). Our reported method [2] was applied to 100 pg of the DNA adduct, N7-(2′-hydroxyethyl) guanine, spiked into DNA. Previously we detected 100 pg of such spiked analyte where sample cleanup relied on SPE. However, the chromatograms displayed additional, non-analyte peaks, and this chemical noise was too severe for reliable detection of 10 pg of such analyte. Further purification here by HPLC of the 100 pg sample leads to a very clean GC–EC-MS chromatogram, as shown in Fig. 1. Such use of HPLC also extends the method to the 10 pg level as shown by the inset in this figure.

This result encouraged us to use satellite HPLC in a procedure under development for the detection of an analogous analyte, O^6-methylguanine. We had been working for over two years to accomplish the measurement of this analyte at the 10 ppb level in DNA by derivatization/GC–EC-MS, while relying on the use of multiple SPE steps in conjunction with derivatization steps to achieve sample cleanup. The two years were filled with frustration due to irreproducible losses and interferences. Switching to satellite HPLC for each of three SPE steps in the method fairly quickly yielded a rugged, successful method (a manuscript is in preparation).

Thus, satellite HPLC is an attractive technique for sample cleanup in trace analysis. An additional advantage to those cited above is that it may be set

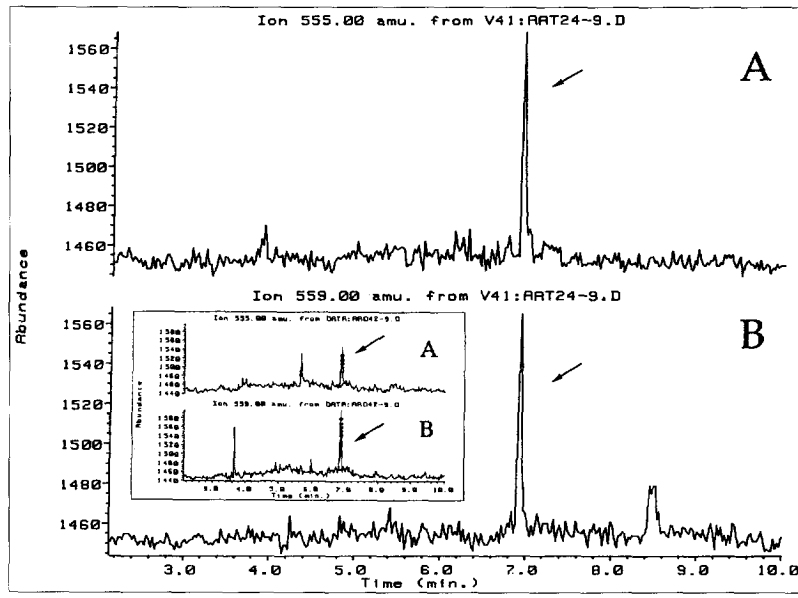

Fig. 1. Detection by GC–EC-MS of 100 pg each of N7-(2′-hydroxyethyl)guanine and an internal standard spiked into 100 μg of DNA, using the procedure reported before [2], plus an additional purification of the final sample by satellite HPLC: Microsorb C_{18} column, 15×0.46 cm, Rainin Instrument Co. (Emeryville, CA, USA); methanol–water (80:20), 1 ml/min; t_R=9.3 min. Sample injections were 30 min apart. The collected sample (2 ml) was evaporated under nitrogen, redissolved in 50 μl of toluene, and 1.0 μl was injected for GC–EC-MS. A=analyte; B=N7-(2′-hydroxyethyl-d_4)guanine, the internal standard, where both were derivatized as described [2]. Inset: similar detection of 10 pg each of this analyte and internal standard spiked into DNA; the actual amount of final derivative of the analyte injected was 50 fg.

ELSEVIER

Journal of Chromatography A, 742 (1996) 299

JOURNAL OF CHROMATOGRAPHY A

Book Review

Aqueous Biphasic Separations Biomolecules to Metal Ions, edited by Robin D. Rogers and Mark A. Eiteman; Plenum Press, New York, NY, 1995, viii+191 pp., price US$79.50, ISBN 0-306-45019-4.

Aqueous two-phase (biphasic) systems have been extensively used for separation and analytical studies of biomaterials since Albertsson developed the technique in the 1950s. The idea of this book was conceived in the Symposium on aqueous biphasic separations held at the 207th American Chemical Society National Meeting (March 1994, San Diego, CA, USA). This symposium gathered USA and international experts and opened scope of two-phase partitioning applications not only for biomolecules, organelles or cells separation but also for metal ions.

Recently, some research groups involved in the development of applications of two-phase partitioning have paid special attention on separation of metal ions by such systems. Their experimental work has shown that this separation also has a potentially remarkable utility for removal of metal pollutants from aqueous media. The main novelty of this book consists in introducing this matter as a recent and arising application of phase partitioning beyond the best known basic or biotechnological uses for separation of biomaterials.

The book consists of theoretical or experimental reviews which fundamentals and applications of the aqueous biphasic systems. After two introductory chapters on the general applications of biphasic separation to metal ions and biomaterials, there are some others devoted to basic principles dealing with hydrophobic and charge effects, surfactant systems and the interfacial events involved in two-phase separations. A group of articles are devoted to the basis [mass transfer, temperature-induced separations, protein refolding and poly(ethylene glycol) (PEG)–protein interactions] or applications (partitioning of protein–PEG conjugates and recombinant proteins) for the extraction of biomolecules by aqueous phase partitioning.

The three remaining items approach the extraction of metal ions either by PEG–salt two-phase system or by affinity partitioning, and one is specifically devoted to the separation of actinides. Such extraction offers an interesting potential for an effective, non toxic and non inflammable, organic solvent-free extraction of metal ions, of doubtless interest for environmental purposes.

This volume is a valuable issue not only for the broad audience of scientists working on phase partitioning, but also for those who feel interested in the potential applications of this technique. Thus, many chemists and engineers who are now entering the field can find in this book an up-to-date information on the current state of understanding and research on aqueous biphasic separation. This book is the kind of contribution needed for creating the multidisciplinary atmosphere of collaboration required for achieving the broadest applications of aqueous biphasic separations to work out production or environmental questions.

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