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Letter to the Editors

On the need to retain unregulated choice of high-performance liquid chromatography columns

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Recollection of their favorable experiences with various RPLC columns was on the minds of those who attended the oral presentations on the ''EU reference C_{18}-bonded silica column'' during HPLC'97, in Birmingham (UK), by a group lead by Dr. K.K. Unger (University of Mainz, Germany) and Dr. R.M. Smith (Loughborough University of Technology, UK) [1]. While all members of the audience may not have known exactly how highly reproducible commercial columns are at present, they did know from their own experience that RPLC is a highly reproducible, yet very flexible method of analysis due in part to the variety of available C_{18} columns. The spirited public discussion that followed the presentations on the use of a single reference column to standardize all C_{18} RPLC separations was mainly opposed to the concept. Some discussants expressed dismay at the potential restrictions that seemed inevitably linked with the required use of a single reference column. Some questioned the political boundary around the reference column development. Some noted that tests, the results of which revealed negative characteristics of the reference column, were deemed unreliable and were eliminated [2,3]. There was particular concern that the reference column might be mandated for regulatory analyses by the food and pharmaceutical industry. When the discussion became polarized, the participants requested that a vote be taken on whether there should be such a reference column. An overwhelming majority of the several hundred participants, questioning the need for such a column, voted against the very idea of a reference column [4]. Unfortunately, this was the only public debate on an issue that could bear heavily on the future of analytical chromatography. The initial declaration of intent at HPLC'93 and the status report at HPLC'97 [1] were never elucidated through scientific publications except for those [5,6] which are a mere rephrasing of the report [1]. Both the scientific validity of the goals of the reference column development and the soundness of the approach have yet to be subjected to a review process involving affected scientists.

Unger claimed [6] that the certified HPLC reference column will simply be like an ISO certified reference material [7] and will serve the same three purposes as such materials in an ''*evaluated, validated and documented test procedure*''. It could be used to check the performance of HPLC instruments, to calibrate HPLC instruments, and to validate the HPLC end determination step of ''*any analytical procedure*''. It can be demonstrated that the reference column cannot fulfill these goals. Purposes one

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and two relate to instrument validation, a process in which most tests are performed without a column [8,9]. Since columns may be associated with artefacts [10], and are limited to certain conditions (e.g., a reference C_{18} column would be inappropriate for checking normal-phase separations, or analysis made with narrow-bore columns or columns packed with small, low-porosity particles, etc.), other rigorous tests are used which do not involve columns. For those tests in which a column is required, there is no need for a special reference column [9]. The third purpose concerning validation of any HPLC analytical procedure should be given careful consideration. In public presentations as in private conversations, German and other European analysts indicate that unskilled analytical chemists from certain lesser developed countries fail to properly validate analyses. If they use the reference column, these individuals would be able to produce reliable analyses because all would be tested every time on their ability to achieve the proper separation of a standard mixture on a reference column [1]. It is highly unlikely that this paternalistic approach would improve the skill of an incompetent analyst. And what about the skilled analyst who must change his system in order to use a reference column to fulfill prescribed requirements, to be obliged to validate again his analytical work?

Over the last thirty years, alkyl-bonded silica columns have matured into a well-defined, highly precise, and most successful analytical tool. A vast majority of the HPLC analyses are carried out in the RPLC mode, often using an alkyl-bonded silica as the stationary phase. The number of C_{18}-bonded silica columns sold yearly, worldwide, is estimated to be nearly one million [1]. Recent work demonstrated that the reproducibility of retention times and peak symmetry data on C_{18}-bonded silica columns is now excellent [8,11,12]. Due to considerable improvements in the industrial synthesis of these packing materials, replacing a column by another one of the same brand but from a different lot will give essentially the same separation. Only minor fluctuations of the retention factors (1 to 3% for all compounds), the separation factors (a few tenths of a percent), the peak efficiency (2 to 6% for neutrals, 2 to 20% for basic compounds) and the resolution (a few % in most cases) could take place [8,11]. With the ability to achieve such high performance reproducibility, who needs a certified reference column, especially if it does not exhibit the same proven record?

RPLC packing materials are made with porous silicas prepared from different silicon products, using different processes. They are bonded with different reagents, following different procedures. The packing materials obtained are similar and, in broad terms, the retention mechanisms involved are the same. However, the detailed balance of the contributions of these mechanisms may differ in important ways on different brands of C_{18}-bonded silica columns. This explains the variety of the retention patterns that can be obtained for a given mixture; replacing an RPLC column of a certain brand by a column of a different brand leads to significantly different separations. Intense work by several independent groups [13–16] has led to a considerable improvement in our understanding of the intricacies of the interactions between the different retention mechanisms involved in RPLC [17]. This very complexity is another feature of RPLC that explains its overwhelming success in the chromatographic analysis of almost all compounds soluble in polar solvents or mixtures of these. Although analysts long considered it a drawback, we now know that it is a great advantage. If, during the development of a new method, it is recognized that a required separation is impossible under the conditions and with the column selected initially, this separation will probably become possible on another RPLC column, packed with a different material. With such a rich complexity, what can a reference column bring which is not covered by an array of commercial ones? How can it help in characterizing a multidimensional space?

Work is progressing on the reference column [18]. It is time to address this issue in public. The scientific community should be prepared for the day when the reference column will be commercially available and an EU directive prescribes its use for regulatory analyses [2,3]. Following a recent political strife due to the discovery of mismanaged projects and other embarrassments, the Commission of the EU is currently being overhauled. Chromatographers can only hope that this reorganization will result in a lesser appetite for regulations constraining scientific activities. The ideal outcome would be the aban-

donment of the reference column project. We strongly recommend this decision.

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