Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 817 (2005) 77–80
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

96-Well solid-phase extraction: a brief history of its development

Richard F. Venn[a,*], James Merson[b], Susan Cole[a], Paul Macrae[a]

[a] *Department of Pharmacokinetics, Dynamics and Metabolism (PDM), Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent CT139NJ, UK*
[b] *Department of Biology, Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent CT139NJ, UK*

Received 10 March 2004; accepted 17 August 2004
Available online 25 September 2004

Abstract

This communication describes the invention and further development of the first 96-well solid-phase extraction system and the original purposes to which it was put. We also describe the adaption of this system for bioanalysis of pharmaceutically active small molecules and the needs underlying it. The system has become a world-wide standard for high-throughput bioanalysis and has been extended by others to include, for example, disk-phase extraction and supported liquid-liquid extraction, as well as 384-well systems. The factors that enabled this leap forward in productivity are discussed.

Keywords: Solid-phase extraction; High throughput; Bioanalysis; SPE systems

Contents

1. Introduction

This brief communication describes the development at Pfizer's Sandwich UK laboratories of the 96-well solid-phase extraction format for bioanalysis that occurred in the early 1990s. It describes the reasons for this development as well as the environment that enabled it.

In the late 1980s Pfizer's research laboratories in Sandwich, UK, were investigating a number of compounds as potential protease inhibitors for use as agents active against viral infection, including HIV-AIDS [1]. In order to determine the effectiveness of these enzyme inhibitors, peptides would be incubated with the proteases in question together with various concentrations of the potential protease inhibitor. After incubation, the effectiveness of the inhibitor was assessed by determining the concentration of one of the peptides released by cleavage of the Tyr-Pro bond in the peptide using an immunoassay. These incubations were carried out in the Biology department in their standard 96-well format.

In order to increase throughput and efficiency for this assay, a collaboration between members of the Biology and the Drug Metabolism departments was started, initiated by the Biology group who wanted assistance in separation and analysis of these cleavage products from the peptides. Solid-phase extraction (SPE) was extensively used in the Drug Metabolism department at that time for the analysis of new chemical entities and was proposed as a means of extraction and separation. The usual format for SPE then was 1- or 2-mL columns containing 50–100 mg sorbent, usually operating in

* Corresponding author. Tel.: +44 304 616 161; fax: +44 304 616 221.
E-mail address: dick@the-venns.demon.co.uk (R.F. Venn).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.08.029

banks of 10 or 12 columns with a vacuum tank, each column discharging after washing into 5–10 mL collection tubes.

This methodology was adopted as appropriate for the separation and extraction of the cleaved peptide products from the incubation prior to final analysis. However, the biology department required a 96-well format to match their incubation and pipetting systems as well as to increase the throughput for screening their compounds. This however did not exist and as a result, a 96-well "assay tray assembly" [2] was invented and patented. This "assay tray assembly" was a standard shallow 96-well plate that was modified to allow a small amount of solid-phase extraction material to be packed in it. This enabled low-volume parallel automated solid-phase extraction to be carried out rapidly.

This assay tray was used successfully for the protease inhibition assay to separate the peptide product from the precursor, prior to the non-selective immunoassay technique used for quantification of the peptide. The plate used was a modified normal 96-well plate of standard depth, holding a total volume of about 200 μL and 25 mg sorbent. Pfizer at that time worked with a local moulding company to develop and then produce sufficient numbers of the modified plate.

In 1990 the Drug Metabolism Department in Pfizer took delivery of its first triple–quadrupole mass spectrometer. This instrument was bought specifically to enable bioanalysis of a Pfizer compound in clinical development at that time, Abanoquil (UK-52,046). This was difficult to analyse by other means and a very sensitive method was required (low pg/mL) that also needed to be selective—there were at least four circulating metabolites. A method was developed and validated for this compound that was very sensitive—a lower limit of quantification (LLOQ) of 10 pg/mL from whole blood was achieved [3]. As use of this instrument increased and further instruments were bought, it became apparent that due to the selectivity of the instruments, short chromatographic run times of only one to two minutes were necessary to achieve the sensitivity and selectivity required. Thus the instruments were idle much of the time, waiting for sample preparation to be completed. One analyst was perhaps capable of preparing one batch of, say 50–60 samples in a day using the then-available 10- or 12-place cartridge/column SPE systems available. This would entail 5 or 6 separate extractions of 10–12 SPE columns on one apparatus, each taking 20–40 min or more if the method was complex or if columns blocked. Thus one analyst would be able to prepare one analytical run of 50–60 samples per day and this batch would take perhaps three hours to complete on the LC–triple–quadrupole mass spectrometer system. It was difficult to run more than one method or compound on each LC–MS–MS system, due to the necessity of changing analytical parameters. Thus a large capital investment could be idle much of the time unless more analysts were able to prepare samples. For example, we developed a method for the analysis of sampatrilat by derivatisation–SPE–LC–MS–MS, a method which required SPE, derivatisation with BF_3-methanol, a second SPE step followed by LC–MS–MS. Throughput was a mere 30 clinical samples per day [4] for four days a week. Our mass spectrometer was at that time therefore very much under-used.

At that time the rationale for buying these expensive instruments was to yield the sensitivity and selectivity required. As their use increased, however, the increased throughput due to the short run times became the focus of attempts to increase their efficiency by running more samples on them in any given time. This desire to utilise the instruments more effectively led to a critical appraisal of all stages of bioanalysis, with the resultant identification of the sample preparation (SPE) step as a major bottleneck.

Having identified SPE as a bottleneck, members of the Clinical Assay Unit of the Department of Drug Metabolism recalled the earlier Biology department's SPE assay tray and embarked on a project to redevelop this into a format that would suit the requirements of ultra-trace bioanalysis. The important changes that would have to be made would enable a number of characteristics of clinical bioanalysis:

1. sample volume would have to increase from 100–200 μL to 1–2 mL—a 10-fold increase.
2. sorbent bed mass would have to increase to 100–200 mg—a five-fold increase.
3. there would have to be zero cross-contamination from well to well. Less critical in the protease inhibition assays, clinical bioanalysis requires high accuracy and precision and cross-contamination could not be tolerated.

We enlisted the help of Porvair Sciences (Porvair Sciences Limited, Shepperton, UK: www.porvair-sciences.com) to design and build the system, which went through several stages of development. At first, we used the original shallow-well plates with the addition of separate drip-spouts (to prevent cross-contamination) inserted under the sorbent bed bottom support. To increase the well volume, we deepened the plates and also had volume extenders added in strips of eight. These extenders were hammered into the top of the plates as a tight fit. Inevitably these early systems were prone to operational difficulties and leaks and blocking and other problems. They did, however, provide a great increase in throughput and we published the first paper on the new 96-well SPE system for bioanalysis in 1996 [5]. This paper described the system with volume extenders. Since then it has been refined and is now manufactured from a single moulding process with no loose parts and is sold by Porvair (who hold the patent rights) as their Microlute^{TM} system. A number of different sorbents are available (e.g. Table 1), and a number of other companies (such as Agilent, Argonaut (formerly IST), 3M Corporation, Varian, Whatman and many others) also manufacture and sell similar systems. Fig. 1 shows the original drawings from the Patent Application and Fig. 2 shows the system as we used it for the extraction of darifenacin.

This 96-well SPE system has become the standard workhorse for bioanalysis throughout the pharmaceutical industry, for development studies, for discovery studies and many others. For example, within PDM at Pfizer's Sandwich site, these 96-well SPE systems are now used for almost all clinical, pre-

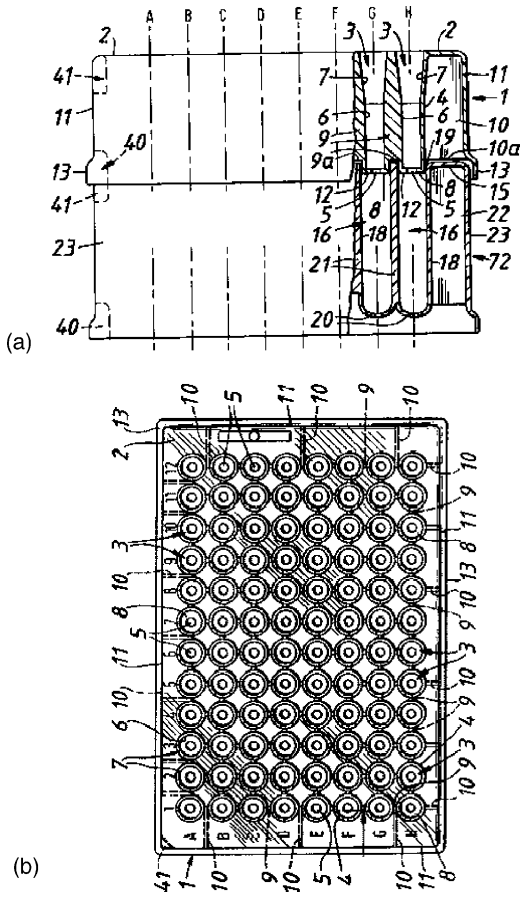

Fig. 1. Showing the drawings of the 96-well assay tray assembly as described in the patent application: (a) cross-section, (b) top view.

clinical and discovery assays and this is reflected throughout the industry both in-house and in contract research organisations.

The number of 96-well SPE blocks used globally is now very high, with a large variety of media available from at least 12 suppliers. These media range from C2, C8 and C18 reversed-phase media to mixed mode and polymeric media. In fact, many suppliers are willing to custom-pack the plates with any desired sorbent or media. Table 1 shows the variety available from one manufacturer in their 50-mg packings. This manufacturer offers packing weights of sorbent from

Table 1
Typical extraction media available in packed 96-well SPE plates

C18 (end capped)	CN
C18	SAX
MFC18	NH2
C8	SCX
C2 (end capped)	PRS
C2	CBA
CH (end capped)	ENV+
PH (end capped)	HCX
PH	HAX
CN (end capped)	Multimode
Si	C8 (end capped)
DIOL	

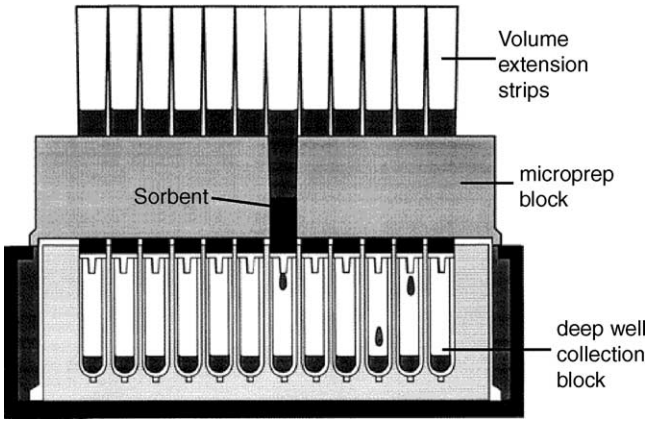

Fig. 2. Showing the 96-well system as used for the extraction of darifenacin with the volume extension strips to increase the available volume to 2 mL.

10–100 mg. Most plates are used in the particle bed format, although disk formats are rapidly becoming very popular. A conservative estimate (based on only one supplier's information) would be that 30,000–50,000 96-well SPE blocks are used annually by the pharmaceutical industry (including CROs) although these figures are difficult to validate. A recent book on high-throughput sample preparation provides much information on 96-well SPE (referring to a selected 25 automated and non-automated methods) as well as many other systems [6]. The number of applications is now very great [6] and is probably largely undocumented, since most pharmaceutical companies currently use them routinely for almost all small-molecule bioanalysis and do not publish the majority of their methods. A number of recent publications have used LC–UV [7], LC–fluorescence [8,9], GC–MS [10] and LC–MS–MS [11–19] systems for analysis and quantification following solid-phase extraction in a 96-well format.

Following on from the development and publication of this 96-well SPE system, a number of other 96-well format analytical systems have been developed. Examples are the disk-based extraction devices, where disks of polymeric sorbents (e.g. Oasis, from Waters Corporation) or polymer-bound silica-based sorbents (e.g. Empore, from 3M Corporation) are provided in the 96-well format. The advantages of such disk-based systems is that elution volumes are very small, allowing sample preparation to avoid an evaporation step with its added time and possible losses due to adsorption. Other examples are of 96-well protein precipitation using the 96-well format in wells or in plates that also provide filtration of the precipitate (from e.g. 3M, Porvair, Whatman, Argonaut) [20]. A further example is of supported liquid-liquid extraction using diatomaceous earth supports in the 96-well format (from e.g. Argonaut).

2. Conclusions and future directions

As is obvious by the take-up throughout the industry of these 96-well systems, they provide a huge advantage over

the previous methods. Combined with the short run-times and selectivity of modern triple–quadrupole mass-spectrometers, such systems allow very rapid method development and operation. It is now possible to achieve analysis of entire clinical studies within a week. The main advantages are that 96 samples can be processed in parallel with very little operator intervention, whereas with the previous sytems an operator would be required to carry out sequential extractions in batches of at most 20 samples. Even when these sequential methods in separate cartridges were automated, each batch would take many hours to run, typically overnight.

It is of interest that this quantum leap in terms of bioanalytical throughput came about through a technological improvement (the atmospheric-pressure ionisation LC–MS–MS) aimed at one unrelated solution (coupling of LC systems to mass spectrometers to improve sensitivity and selectivity). This technology allowed a new way of working and subsequently drove a different agenda (increased throughput). This new way was developed because there was an opportunity for members of the same organisation in different departments to collaborate and work together. There are a number of lessons that could be drawn from this:

- People in organisations should strive to work together and to collaborate. This is often difficult in any setting and in increasingly globalised industries groups who could benefit from such collaborations may be in different continents.
- Within any research setting, long-term employees are extremely valuable. They remember things, methods, ideas from the past and can draw on their experience to facilitate and create new possibilities.
- Cross-departmental fertilisation happens when departments are located in close proximity to each other – preferably within the same building – and when individuals know each other, or can easily make connections to people who can help them.

The 96-well SPE system has led on naturally to 384-well SPE which has been shown to be possible [21,22] although not without its difficulties. It may however be that the 96-well format is preferred since the volume of the 384-well systems is limited and the small size and low extraction-medium mass is likely to cause technical problems such as blocking with real-life samples of plasma and serum. That there are only two current publications on 384-well systems known to us may indicate the problems. The disadvantages are likely to outweigh the advantages, and 384 wells may in fact be too many for most applications. As far as this group is concerned, a 192-well format (or, radically, 200 wells) could be a preferred optimum compromise.

Acknowledgements

Barry Kaye was the inspiration for improving the throughput of bioanalysis in the Drug Metabolism Department's Clinical Assay Unit; Tony Castleman and Richard Ireland of Porvair Sciences never gave up and made the system work, making modifications and alterations along the way.

References

[1] S.M. Cole, P.V. Macrae, J.R. Merson, F.S. Pullen, D.J. Rance, J. Chromatogr. 562 (1991) 67.
[2] J.R. Merson, D. Bojanic, European Patent EP0454315, "An assay tray assembly" Pfizer Limited, 8 April 1991.
[3] B. Kaye, M.W. Clark, N.J. Cussans, P.V. Macrae, D.A. Stopher, Biol. Mass Spectrom. 21 (1992) 585.
[4] R.F. Venn, B. Kaye, P.V. Macrae, K.C. Saunders, J. Pharm. Biomed. Anal. 16 (1998) 875.
[5] B. Kaye, W.J. Herron, P.V. Macrae, S. Robinson, D.A. Stopher, R.F. Venn, W. Wild, Anal. Chem. 68 (1996) 1658.
[6] D.A. Wells, Progress in Pharmaceutical and Biomedical Analysis, vol. 5, "High Throughput Bioanalytical Sample Preparation—Methods and Automation Strategies", Elsevier, 2003.
[7] L.H. Du, Y. Xu, D.G. Musson, J. Chromatogr. B (2003) 794.
[8] P.H. Zoutendam, J.F. Canty, M.J. Martin, M.K. Dirr, J. Pharm. Biomed. Anal. 30 (2002) 1.
[9] C.Z. Matthews, E.J. Woolf, B.K. Matuszewski, J. Chromatogr. A 949 (2002) 83.
[10] F. Pommier, F. Boschet, G. Gosset, J. Chromatogr. B 783 (2003) 199.
[11] H.Y. Kim, K.Y. Chang, H.J. Lee, S.B. Han, K.R. Lee, J. Pharm. Biomed. Anal. 34 (2004) 661.
[12] P.H. Zoutendam, M. Gavin, M.J. Martin, M.K. Dirr, J. Pharm. Biomed. Anal. 33 (2003) 1073.
[13] L.D. Williams, L.S. Von Tungeln, F.A. Beland, D.R. Doerge, J. Chromatogr. B 798 (2003) 55.
[14] D. Whittington, E.D. Kharasch, J. Chromatogr. B 796 (2003) 95.
[15] L.Y. Yang, R.P. Clement, B. Kantesaria, L. Reyderman, F. Beaudry, C. Grandmaison, L. Di Donato, R. Masse, P.J. Rudewicz, J. Chromatogr. B 792 (2003) 229.
[16] J. Lee, J. Son, M. Lee, K.T. Lee, D.H. Kim, Rapid Commun. Mass Spectrom. 17 (2003) 1157.
[17] A. Schuster, S. Burzawa, M. Jemal, E. Loizillon, P. Couerbe, D. Whigan, J. Chromatogr. B 788 (2003) 377.
[18] C.R. Mallet, Z.L. Lu, R. Fisk, J.R. Mazzeo, U.D. Neue, Rapid Commun. Mass Spectrom. 17 (2003) 163.
[19] A.Q. Wang, W. Zeng, D.G. Musson, J.D. Rogers, A.L. Fisher, Rapid Commun. Mass Spectrom. 16 (2002) 975.
[20] R.A. Biddlecombe, S. Pleasance, J. Chromatogr. B 734 (1999) 257.
[21] G. Rule, M. Chapple, J. Henion, Anal. Chem. 73 (2001) 439.
[22] R.A. Biddlecombe, C. Benevides, S. Pleasance, Rapid Commun. Mass Spectrom. 15 (2001) 33.