Identifying Inherent Contamination in Deep Well Microplates

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Deep well microplates are an important class of functional labware used for sample preparation, compound storage, mixing, transport and fraction collection in many laboratories. They can also be used in many commercially-available autosamplers and come in a range of sizes and plate formats, the most commonly used being 96-well and 384-well plates made from polypropylene.

In previous independently run evaluation reports (1,2) in 2005 and again in 2013, it was shown that commercially available deep well microplates can harbour significant levels of extractable compounds which come from use of lower grade polypropylene polymer.

In this review additional findings by Weikert et al. (3) will be considered. In this report the researchers find significant levels of contamination in samples of many commercially available microplates.

The academic study by Howland performed at Kent University broadened the scope of previous work and tested not only "natural" polypropylene microplates, but coloured and black plates and a much wider range of manufacturer's products than before. This study, gives data on a large range of microplates from numerous manufacturers based in Europe, USA and China. Mass spectral data shows that persistent contamination from a range of compounds found in the raw polymer master batch continues to be evident in many of the microplates tested.

Background

The effect of extractables identified in this report is complex and depends on the exact application for which the plate is designed. However, it is clear that long chain hydrocarbon contaminants will certainly cause extraneous fluorescence signals in any spectroscopic work and may even cause false positives in large scale screens looking at apoptosis or stasis in whole cells. In forensics, these unwanted small molecules can easily mask drugs of abuse or their metabolites and cause ion suppression, which leads to inaccurate quantification. Scientists using deep well microplates must ensure regular screening of the plates in use if they are to avoid such unwanted contamination. Testing can be carried out in house, alternatively the use of certified plates will ensure a certain quality standard.

D

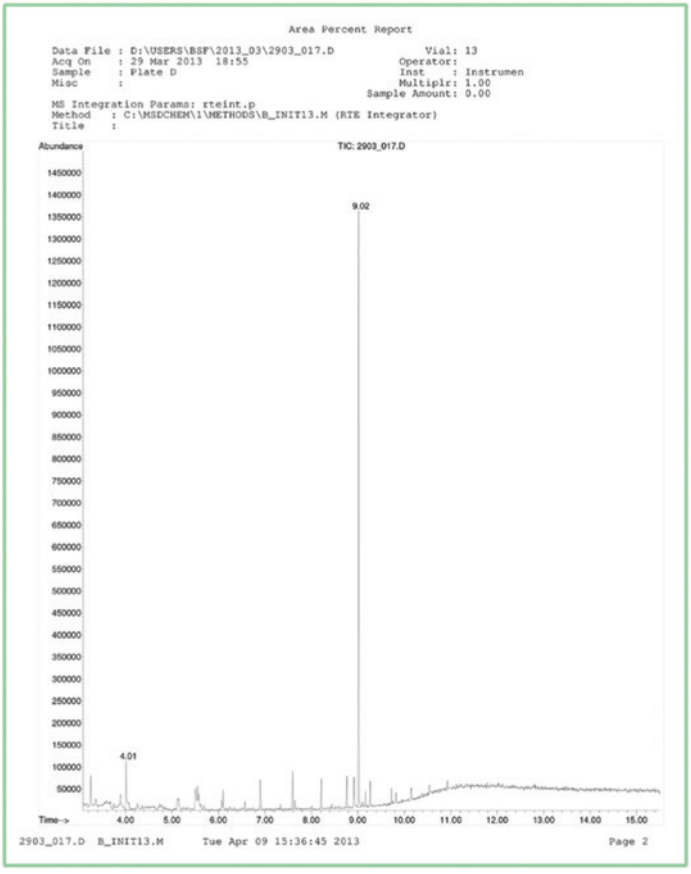

Figure 1: Plate D Porvair 2ml square well plate

E

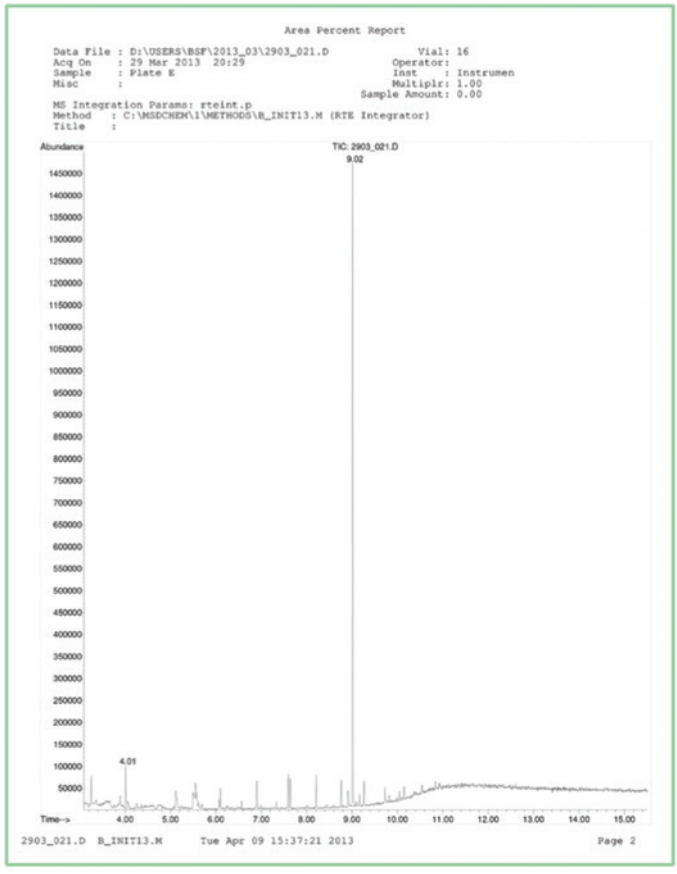

Figure 2: Plate E Porvair 1ml round well plate

F

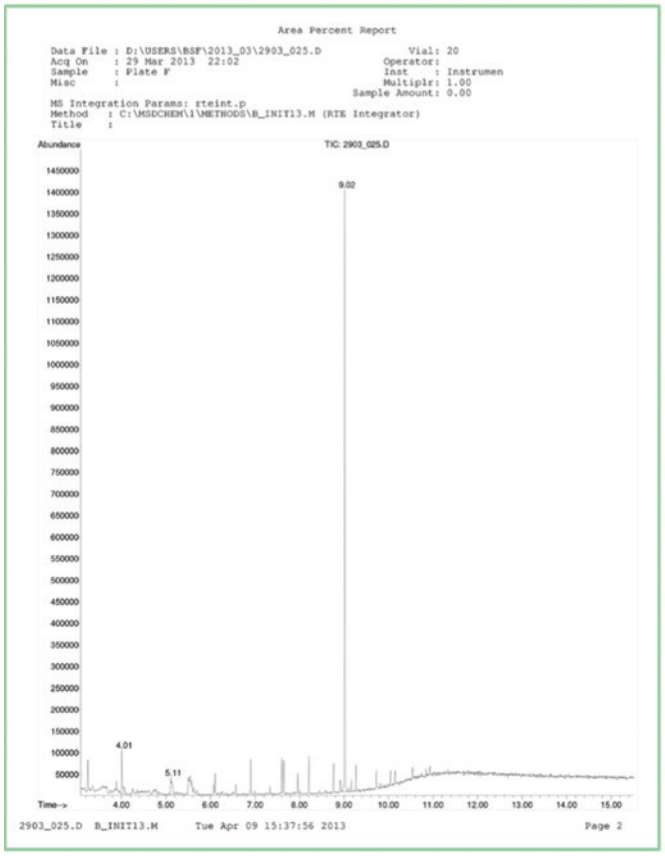

Figure 3: Plate F Porvair 1ml round well black plate

G

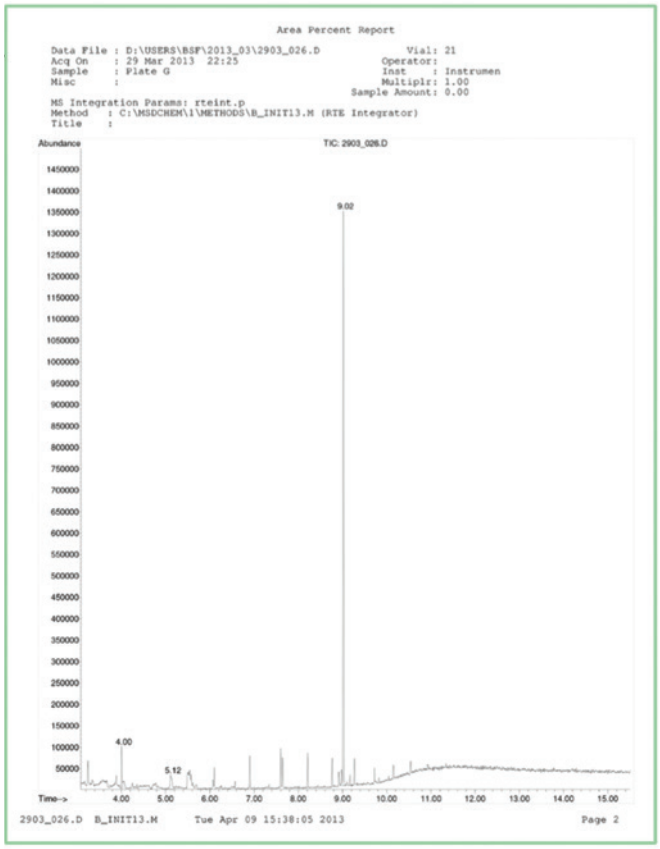

Figure 4: Plate G Porvair 1ml round well red plate

H

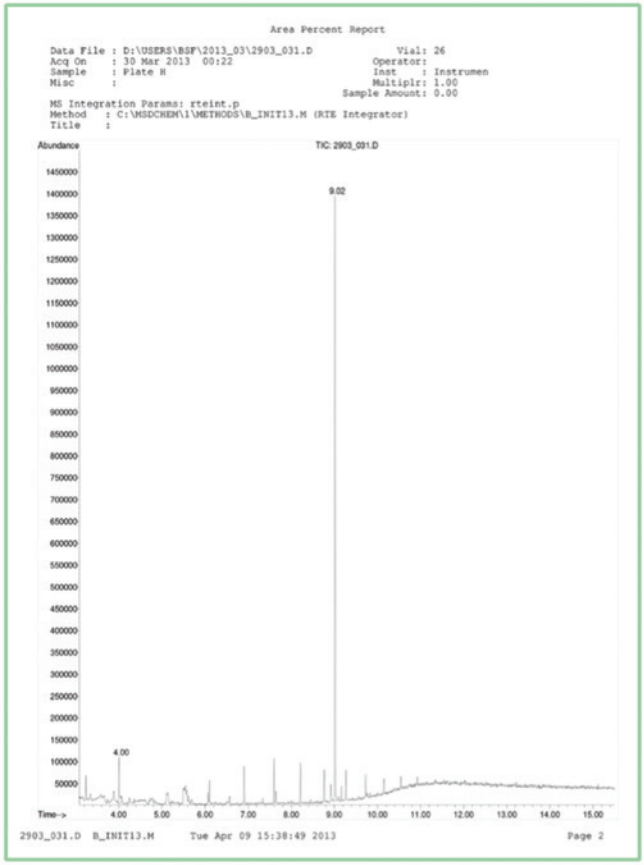

Figure 5: Plate H Porvair 1ml round well blue

A

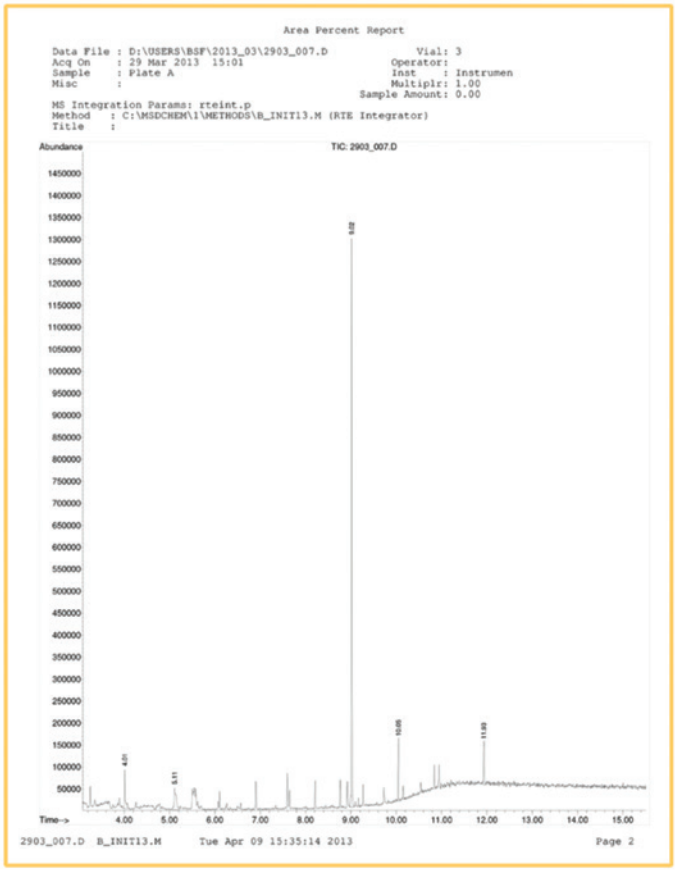

Figure 6: Plate A European 1.6ml low profile plate

B

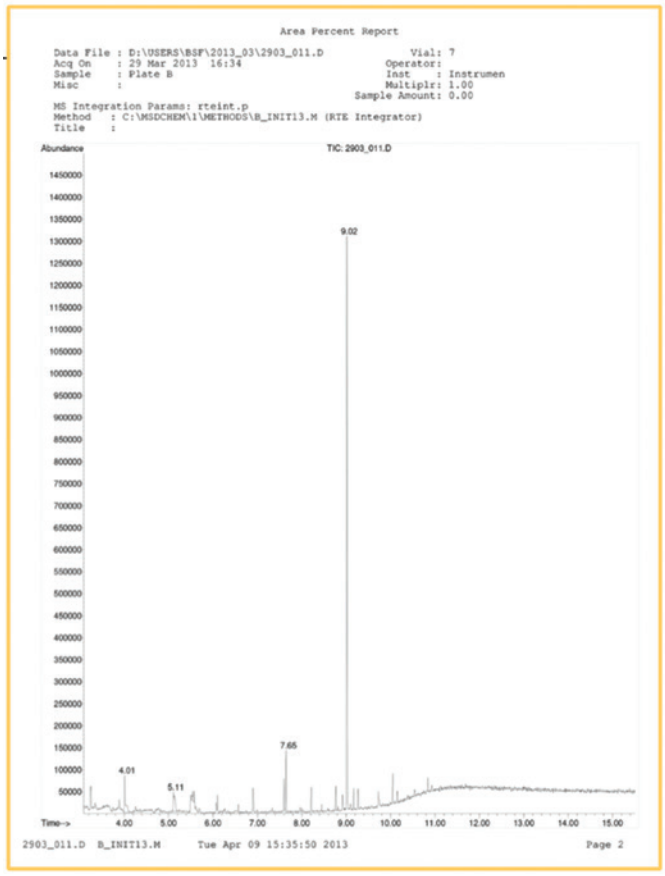

Figure 7: Plate B USA 10ml storage plate

J

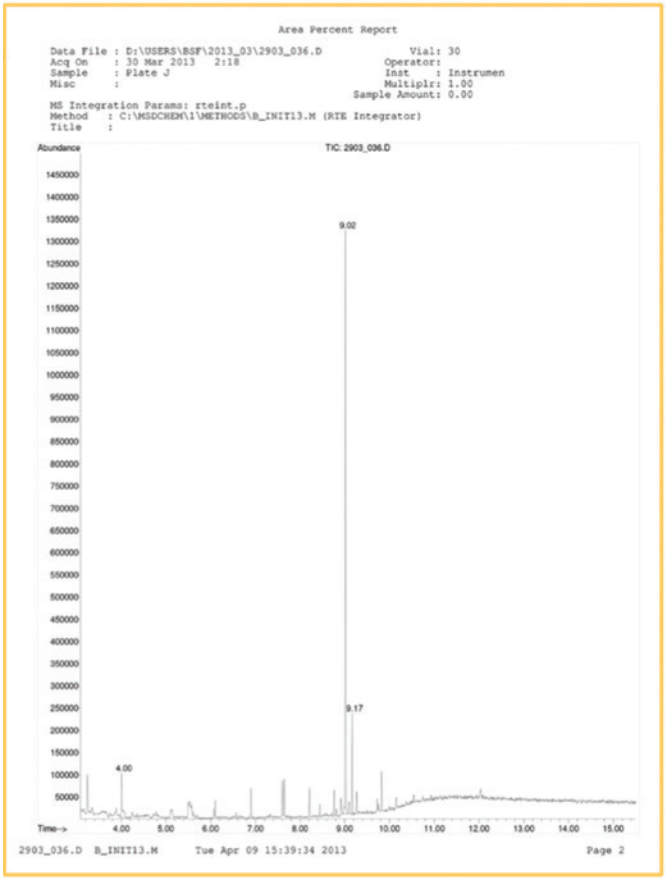

Figure 8: Plate J USA 2ml deep well plate

N

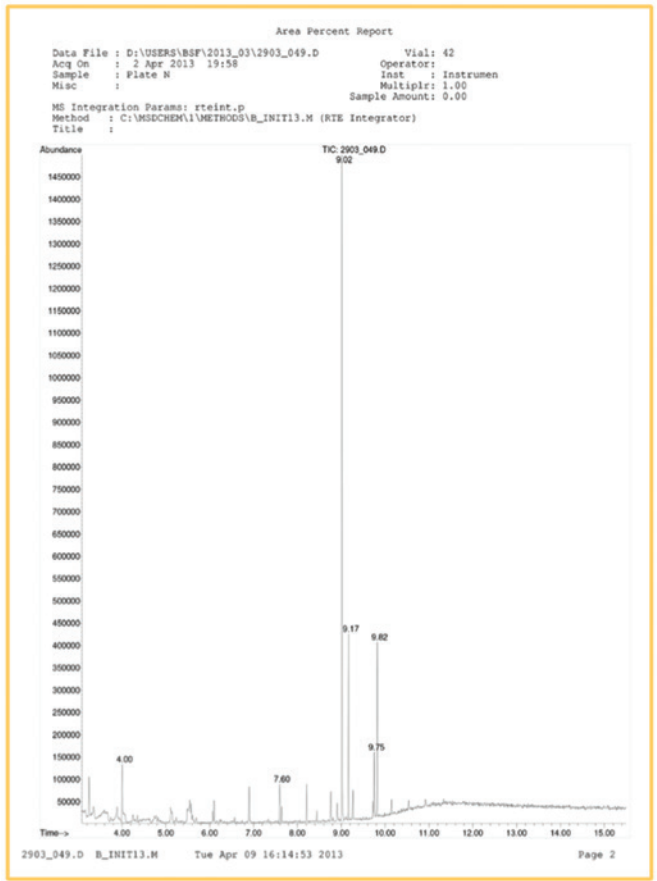

Figure 9: Plate N USA 1ml round well plate

C

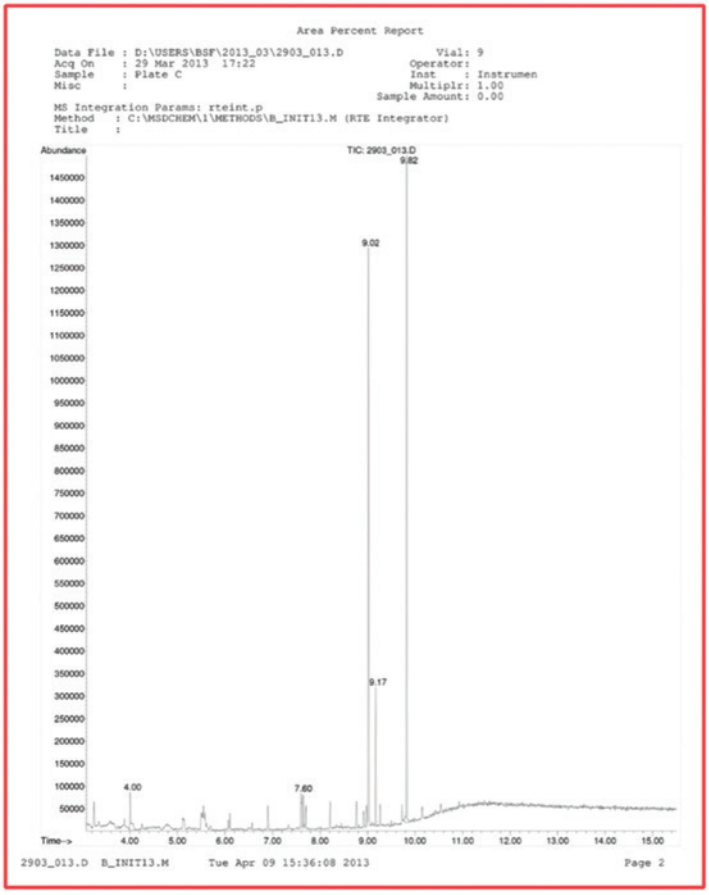

Figure 10: Plate C USA 48 well deep well plate

M

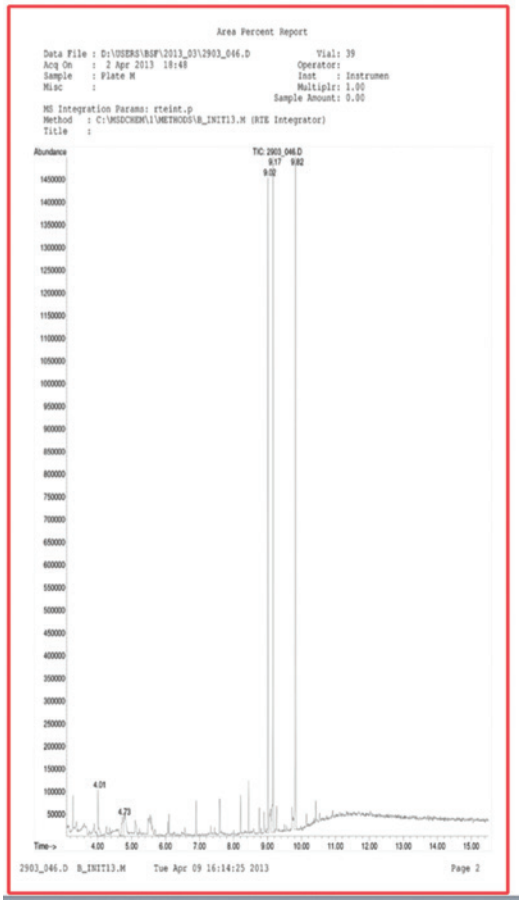

Figure 11: Plate M European 1ml round deep well plate

Q

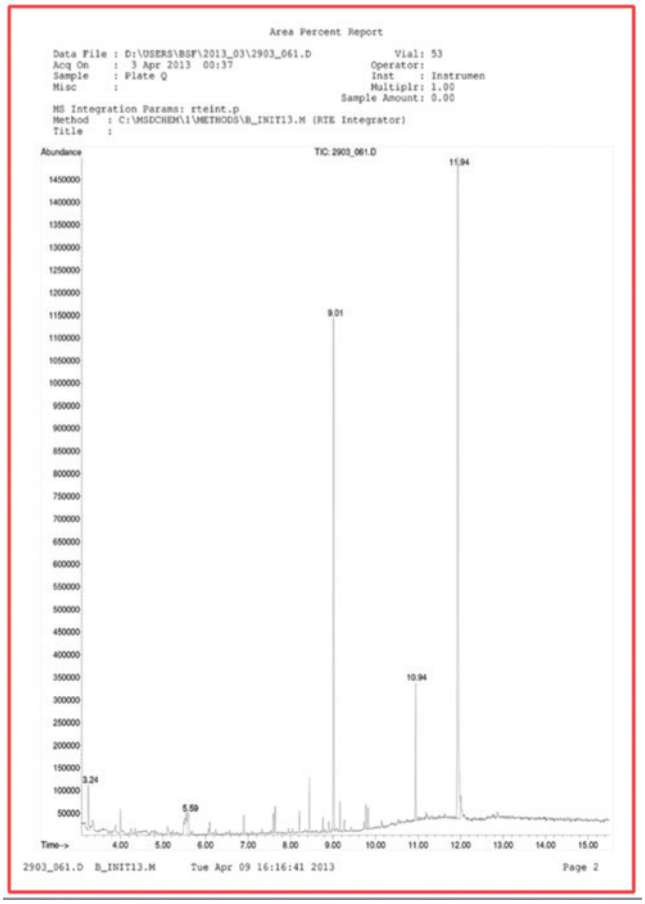

Figure 12: Plate Q USA 2ml square well deep well

Method

In the 2005 study, samples of deep well microplate for testing were obtained from all the major manufacturers. A new unused plate was selected from each batch and subjected to a stream of clean, dry compressed air to remove any particulates that may have accumulated. Testing for polymer leachate and extractable contamination was performed by incubating overnight an appropriate volume of HPLC grade methanol in three wells in each sample plate. The methanol was spiked with 10 µg/ml of Caffeine as an internal standard. The plates were sealed with a friction seal and left to stand overnight.

After overnight incubation, 1 µl aliquots of each well sample were subjected to analysis on an Agilent GC-MS system using splitless injection at 250°C.

Separation was performed on an Agilent HP-5MS 0.25mm x 30m x 0.25um capillary column using the following temperature gradient.

Initial Temp 70°C Hold 2 min

Ramp 30C / min

Final Temp 310°C Hold 5 min

Detection was by positive ion EI-MS. Integrated peaks were searched against a NIST98 spectral library (scores over 95 being significant hits in the database). A blank was also run using only caffeine-spiked methanol held in silanised glass overnight which had not contact with any polymer.

In order to simplify the full data set here, results from each of the three wells per plate tested have been combined and averaged.

Results

Plates showing no measurable contamination are shown in green. Some minor contamination, defined as only one contaminant and not necessarily in all three wells tested, are in yellow. The worst performing plates, showing considerable contamination are shown in red. With

Description	I.D.	Results
Porvair 2ml square well plate	D	Extractibles Free
Porvair 1ml round well plate	E	Extractibles Free
Porvair 1ml round well black plate	F	Extractibles Free
Porvair 1ml round well red plate	G	Extractibles Free
Porvair 1ml round well blue	H	Extractibles Free
European 1.6ml low profile storage plate	A	Minor Contaminant
USA 10ml storage plate	B	Minor Contaminant
USA 2ml deep well plate	J	Minor Contaminant
USA 1ml round well plate	N	Minor Contaminant
USA 48 well deep well plate	C	Significant Contaminant
China sourced 2ml deep well plate	I	Significant Contaminant
European Microcentrifuge tubes, black	L	Significant Contaminant
European 1ml round deep well plate	M	Significant Contaminant
USA 2ml square well deep well plate	Q	Significant Contaminant
European 2ml square well, round bottomed plate	S	Significant Contaminant

Plates/Tubes used in this study were from different sources of manufacturers in the USA and Europe.

S

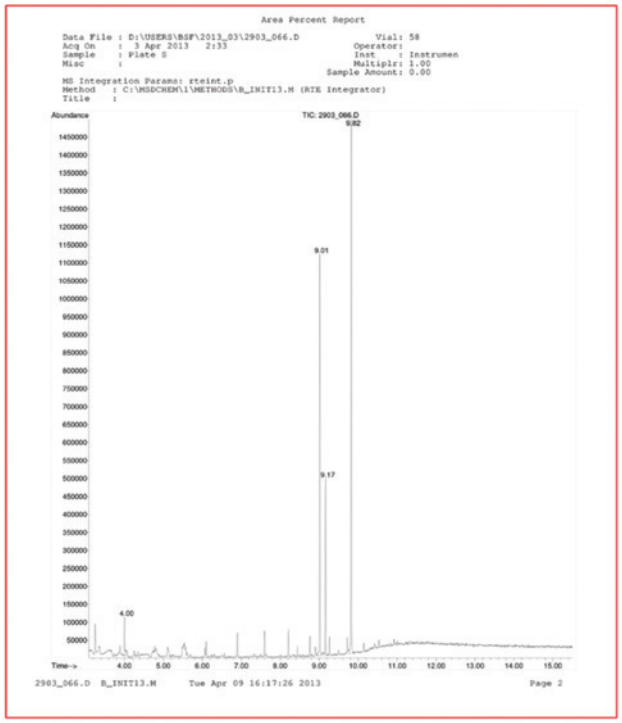

Figure 13: Plate S European 2ml square well, round bottomed plate

the caffeine standard giving a relative abundance of 115000, any potential contaminant used to group a plate into green, yellow or red categories must show an abundance greater than 10000. In the worst case plates, contaminants exhibited relative abundance in excess of 155000 and in one case, off the scale of the integrator.

Contaminant Identification

The extracted contaminants were compared against an NIST database to identify the chemicals involved. These chemicals were shown to be in a group which acts as plastics enhancers and mould flow agents to assist ease of injection moulding.

Other chemicals were apparent in some of the products at lower levels and for the sake of comparison to the original study were not identified in full.

It is likely the above chemicals are incorporated in the polymer mix at an early stage to enhance ease of moulding and fast cycle time of the mould to produce the relevant products. The extra work conducted by the SiO2 team used a more aggressive extraction method involving a 72 hour exposure to acetonitrile and this produced, albeit at lower levels, more identifiable contaminants from some of the polypropylene microplates under test. These included anti-static agents and antioxidants, including the anti-oxidant Tris (2,4-di-tert-butylphenyl) phosphite, slip agents which included glycerol palmitate and glycerol stearate together with the polymer nucleating agent bis-(3,4-dimethylbenzylidene sorbitol diacetal).

Contaminants	Commercial Use
Eucrylamide	Anti-Caking agent, lubricant
Dodecanoic Acid	Mould release agent
Hexadecanoic Acid, methyl ester	Softner, accelerator activator
Octadecanoic Acid, methyl ester	Plastics lubricant
9-Octadecanoic Acid, oleic acid	Plastics lubricant, release agent

Conclusion

The results show that, despite the ready availability of high quality medical-grade polypropylene for deep well microplate production, more than half of the tested plates exhibited some form of contamination; typically, due to extractable compounds leaching out of the plastic. In both previous independent lab testing studies, all Porvair Sciences deep-well plates were shown to be contamination-free.

In the 2013 study, a significant selection of deep well plates from different suppliers were found to be contaminated with extractables. This leads to the conclusion that a low grade polypropylene was used in the microplate production. Such low-grade polypropylene often contains flow modifier additives or "mould release agents" which have been used to ease the manufacturing process and help free the moulded microplate from the mould respectively. The use of methanol in this study is significant. As a polar solvent it is very good at dissolving small molecules and has been shown to extract most non-polymer bound compounds from polypropylene microplates. It is also a very suitable solvent for GC-MS. However, if an even more powerful solvent, such as DMSO, were used for this work, it is likely that even more extractables would be seen.

Implications

With the continuing use of deep well plates for sample introduction in LC/MS analysis in pharma drug discovery, pesticide and biocide development and detection, ADME/Toxicology, forensics & narcotics testing and in neo-natal errors of metabolism studies where small molecule detection at low levels is prevalent, inherent contamination from the labware could pose a significant risk of false positives or assay data corruption in large-scale "hit-to-lead" screens.

Recommendations

It is recommended that deep well microplates used for long-term storage of samples in organic solvents are regularly assessed to check for extractable contamination. Consequently, it is important that this phenomenon is understood before stored compounds or samples are further analysed. Efforts should be made to obtain certificates of purity from the manufacturer during method validation and where this is not possible; extractables testing should be carried out to prevent the risk of contamination of valuable compounds and samples. In the case of Porvair Sciences plates, all the polypropylene used is rigorously tested for extractables and leachates before entering our clean-room mould production. Care is taken to use polyethylene bags which are also extractable free and certificates of analysis can be provided for each batch of product. In addition the Class 10000 clean room production environment ensures that the plates are also DNA/RNA, DNA/RNAase and endotoxin free at the point of use.

References

1. Extractables Testing in Deep Well Plates", Ireland, Castleman et al, Porvair Sciences, 2005

2. Controlling inherent contamination in deep well microplates", Knight & Castleman, Porvair Sciences 2013

3. Plasma-treated Microplates with Enhanced Protein Recoveries and Minimized Extractables Weikart, Klibanov, Breeland, Taha, Maurer and Martin, SLAS Technology, 2017, Vol 22 (1) 98-105