Controlling inherent contamination in deep well microplates

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Introduction

In a previous independently run evaluation report ("Extractables Testing in Deep Well Plates", Ireland, Castleman et al, Porvair Sciences, 2005) we have 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 study, conducted on behalf of Porvair Sciences R&D by the Department of Chemistry at the University of Kent, a further and more detailed study of commercial deep well microplates is conducted. Once again, it finds significant levels of contamination in samples of many commercially available microplates.

This new study broadens the scope of previous work and tests not only "natural" polypropylene microplates, but coloured and black plates and a much wider range of manufacturer's products than before.

The study, carried out in early 2013, 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 you can choose your deep well microplates just from reputable companies who can guarantee the absence of such interfering compounds in their products.

Method

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 10ug/ml of Caffeine as an internal standard. The plates were sealed with a friction seal and left to stand overnight.

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

Separation was performed on a capillary column using the appropriate temperature gradient. Detection was by positive ion EI-MS.

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

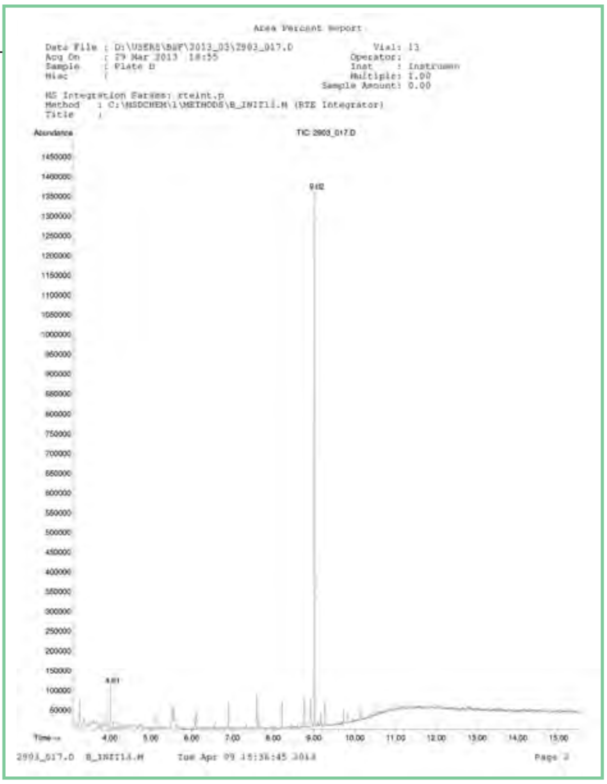
Results

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 in red.

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 starge 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.

D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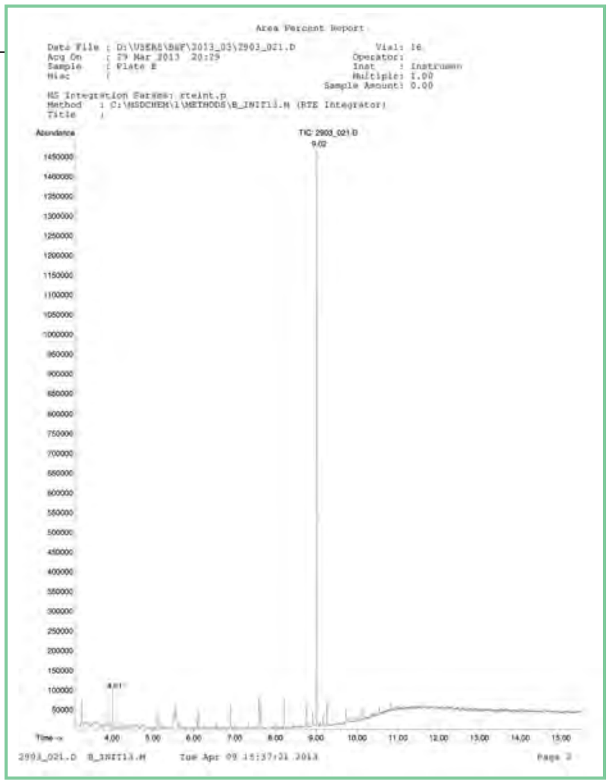
E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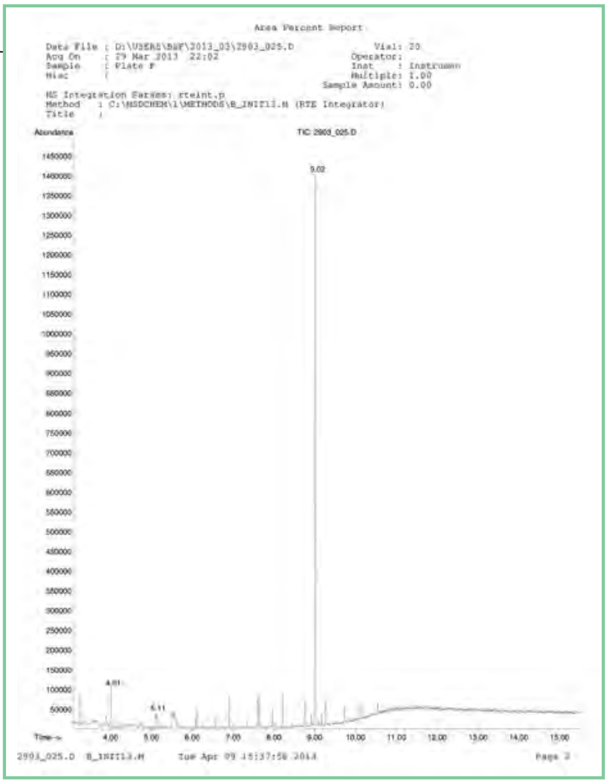
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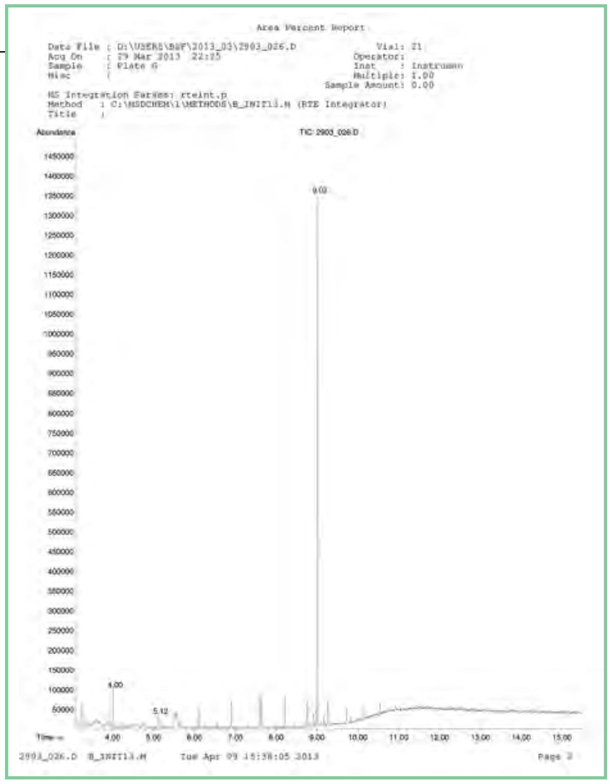
G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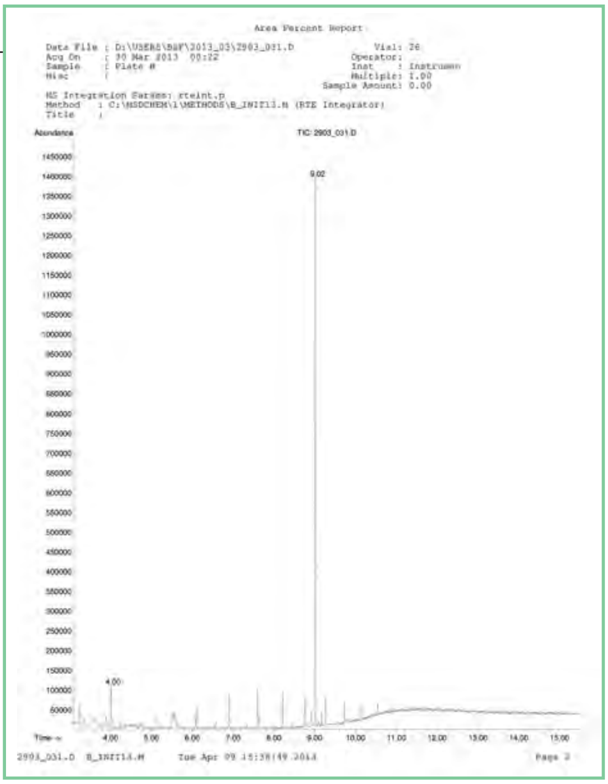

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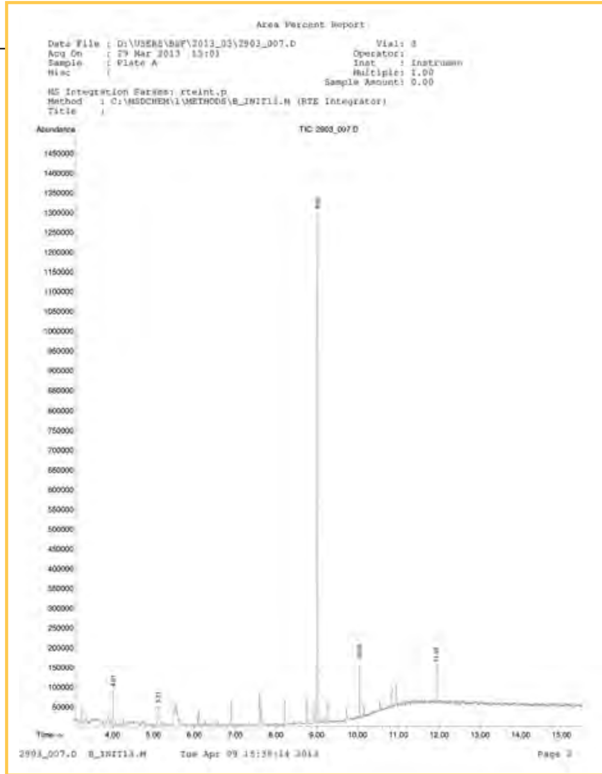

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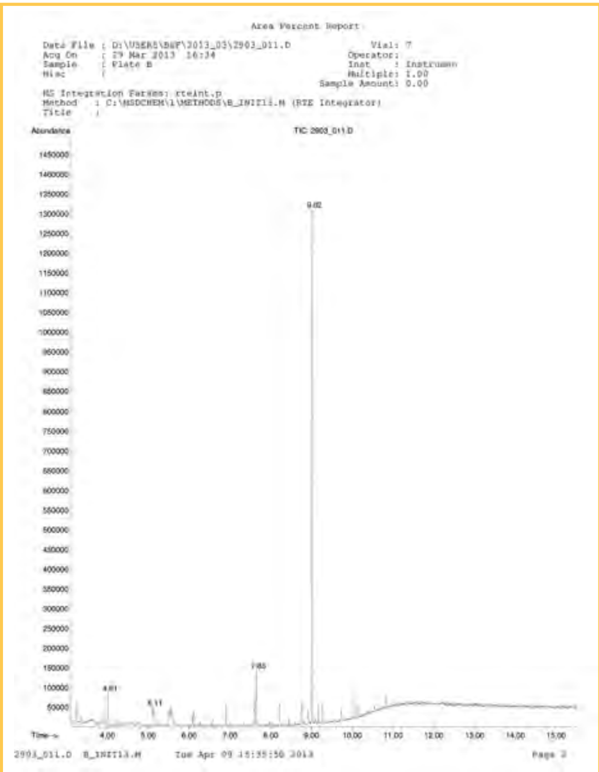

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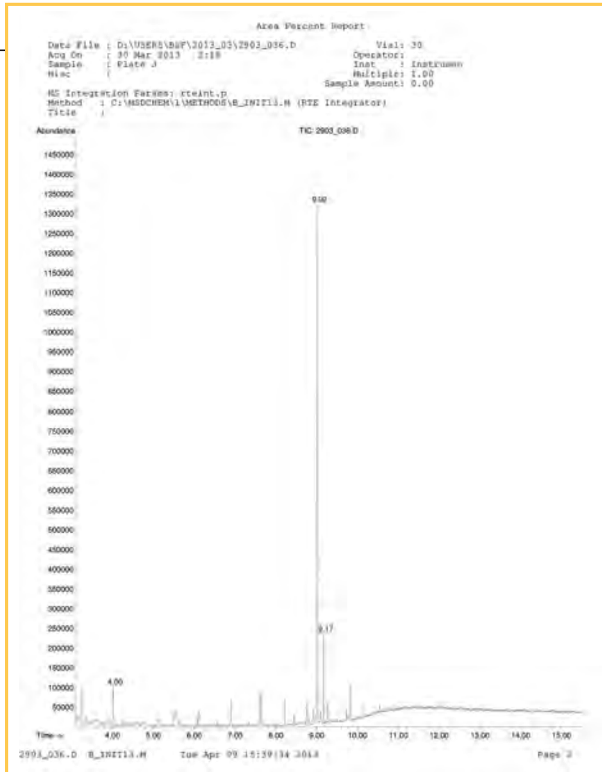

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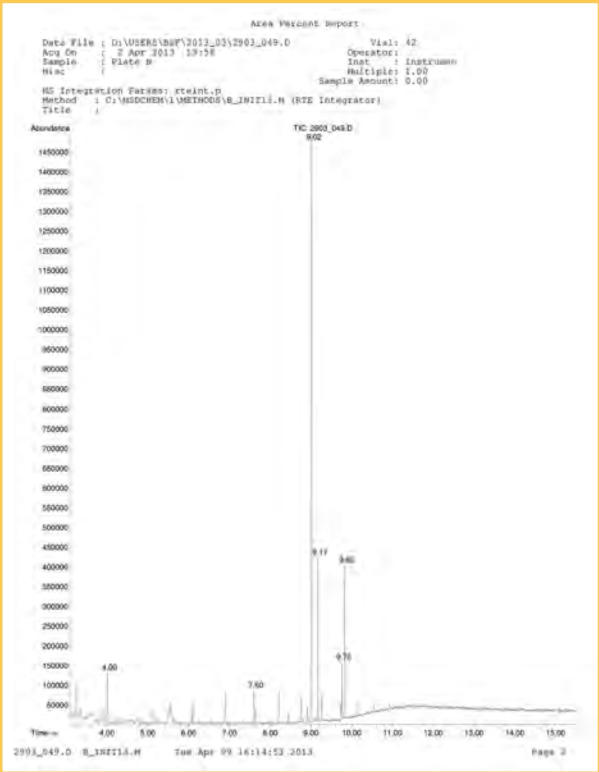

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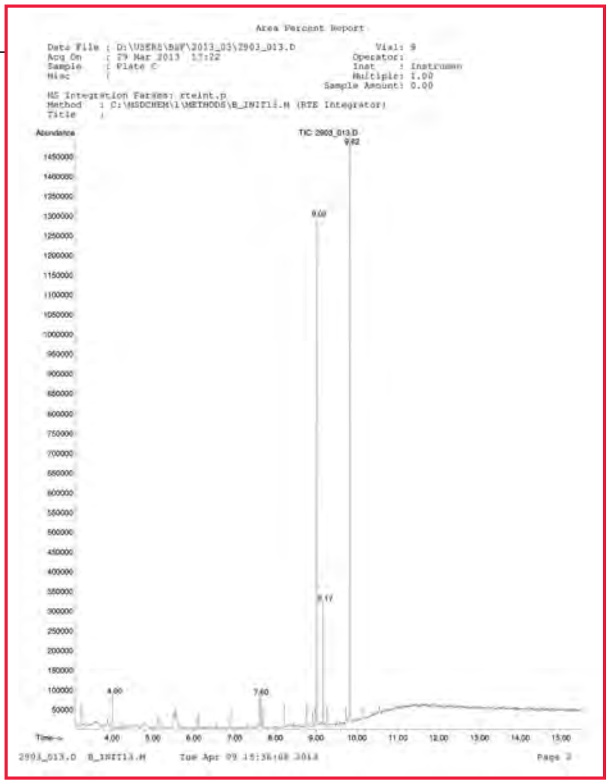

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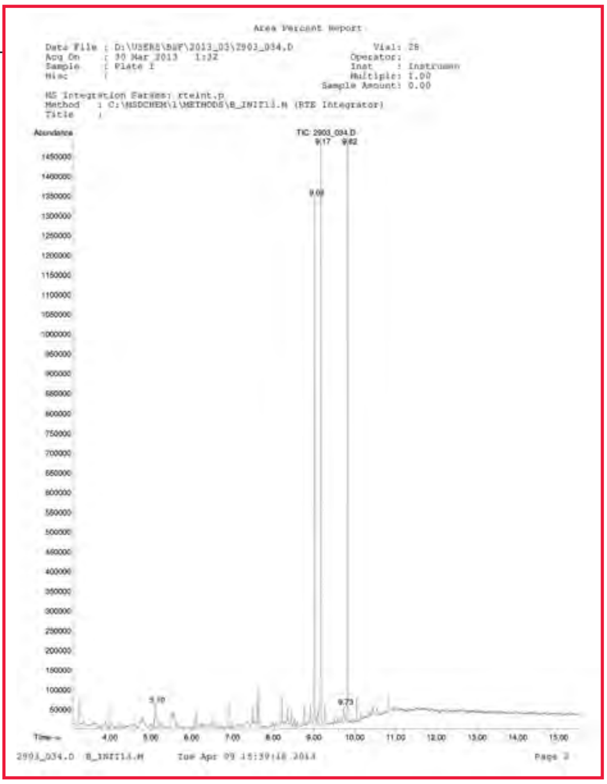

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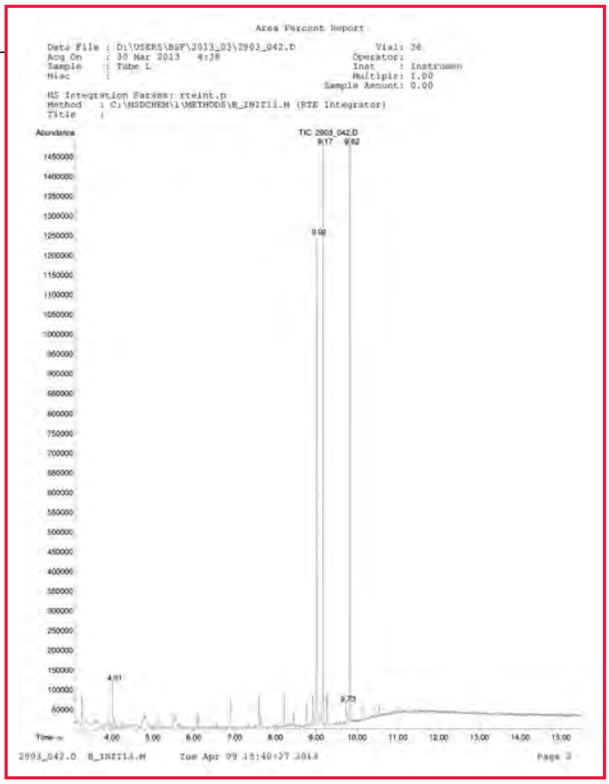

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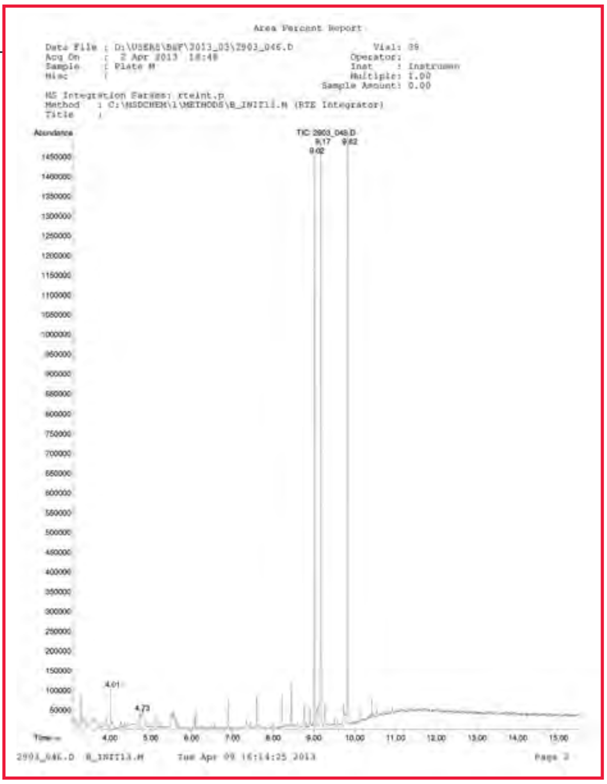
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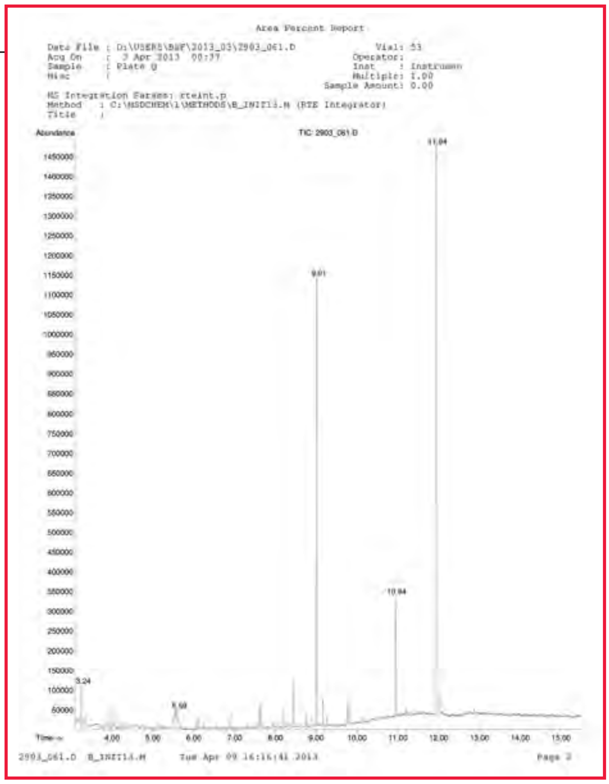
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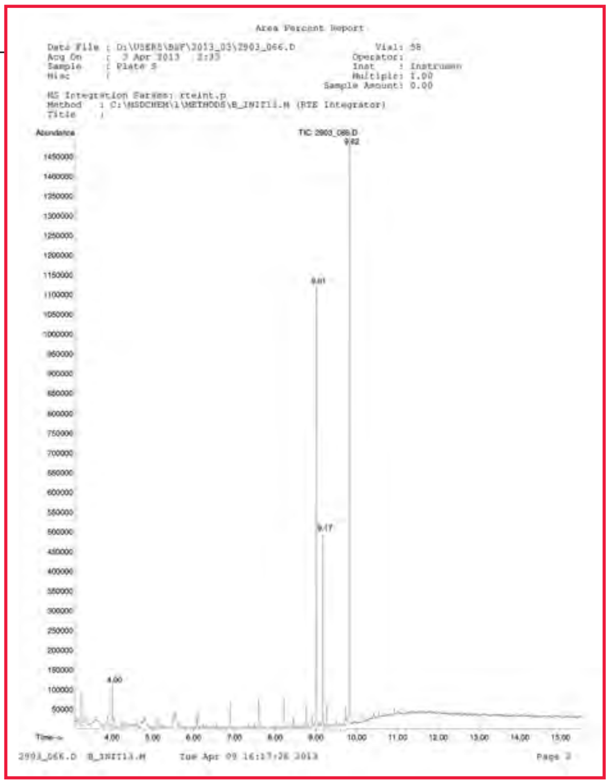
S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Contaminant Identification

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.

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

Other chemicals were apparent in some of the products at lower levels and for the sake of the 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.

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. As in the 2005 independent lab testing, all Porvair Sciences deep-well plates were shown to be contamination-free.

In this 2013 study a significant selection of deep well plates from different suppliers were found to be contaminated with extractables. This leads us to conclude 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 DMSO, were used for this work, it is likely that even more extractables would be seen.

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 phenonomen is understood before your 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.

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