White Paper 21150

Mass Spectral Studies of Polypropylene Chromatographic Well Plates

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Key Words

GC Extraction, Extractables, Silicone Mats, Sensitivity, Well Plates, Polypropylene

Introduction

In a previous independent study, commercially available deep well microplates were shown to harbor significant levels of extractable compounds. It has been proposed, that this is from the use of lower grade polypropylene polymers and/or deforming reagents used during the molding process itself.

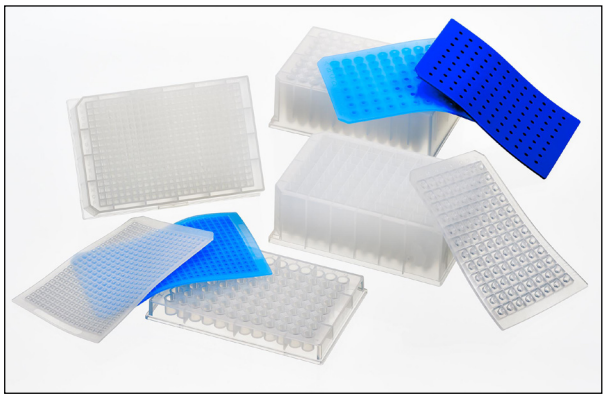
This study has been undertaken to identify low extraction 96-well deep well plates that are suitable for use with high sensitivity chromatographic methods.

Mass spectral data shows that persistent contamination from a range of compounds found in the raw polymer master batch is evident in many of the micro plates 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 signals in any spectroscopic method including UV detection in HPLC. 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 micro plates must ensure regular screening of the plates in use if they are to avoid such unwanted contamination or re-contamination of (expensively) cleaned up samples during storage in well plates.

Testing can be carried out in-house, but this study shows that plates can be pre-tested for their suitability for applications involving injection into chromatographic instruments with mass spectral detection.



Thermo Scientific™ WebSeal™ system for chromatographers.

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Method

A new, unused plate was selected from each batch. Testing for polymer leachate and extractable contamination was performed by incubating (overnight) an appropriate volume of HPLC grade methanol in 12 wells in each sample plate. The plates were sealed and left to stand overnight.

After overnight incubation, 1 µL aliquot of each well sample was 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 (Electron Ionization-Mass Spectrometry).

GC/MS

Instrument:	Thermo Scientific™ ISQ™ Series GC-MS with Thermo Scientific™ Triplus™ AS Autosampler
Software:	Thermo Scientific™ Xcalibur™
Column:	Thermo Scientific™ TraceGOLD™ TG-5MS, 30 m × 0.25 mm × 0.25 µm
Carrier Gas:	Helium
Flow Rate:	1.2 mL/min
Oven Program:	40 °C, hold for 0.5 min; 15° C/min to 150 °C, hold for 1 min; 10 °C/min to 290 °C, hold for 5 min
Inlet Temperature:	250 °C; Split flow: 50 mL/min
Injection Volume:	1 µL splitless
MS Transfer Line:	290 °C
MS Ion Source:	230 °C
MS Detection:	Positive EI; Full scan 50 to 650 *m/z*

Results

A number of candidate plates showed minimal extraction peaks as shown in Figures 1 and 2. A number of these plates have been submitted to stringent certification to allow assurance for their usage in testing MS analysis.

Where a number of extraction peaks were determined antioxidants (stabilizers) were most likely to be found in low levels. Examples are shown in Figure 3 where a single peak extract was identified and Figure 4 where isolation and identification of discrete peaks are determined from the MS spectra.

Where multiple peaks are determined the plate has a range of extractants including antioxidants and cannot be recommended for chromatographic use. An example shown in Figure 5.

External contamination sources should not be ignored and the use of silicones and solvents with the plates can cause leaching and a transfer of silicone oligomers as shown in Figure 6. Discrete peaks for mold release can be seen in Figure 7 for a rejected plate format.

Contaminant Groups

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 mold flow agents to assist ease of injection molding.

It is likely that the above chemicals are incorporated in the polymer mix at an early stage to enhance ease of molding and fast cycle time of the mold to produce the relevant products.

Table 1: Contaminants and their commercial use.

Contaminants	Commercial Use
Eucrylamide	Anti-caking agent, lubricant
Dodecanoic Acid	Mold release agent
Hexadecanoic Acid, Methyl Ester	Softener, accelerator activator
Octadecanoic Acid, Methyl Ester	Plastics lubricant
9-Octadecanoic Acid	Plastics lubricant, release agent
Bis 3, 5, Dimethyl 4-hydroxy Benzenepropionic Acid Ester	Antioxidant

Conclusion

It is possible to select microplates manufactured with high purity polypropylene for use as a certified chromatography injection or storage plate.

Some plates were determined to have limited extraction components and may be used for routine HPLC and other chromatographic techniques (with water samples or limited polar solvent content) if the extraction products are avoided.

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.

Contamination of plates from external sources were also found, which, while isolated, did show that transport, storage and packaging must also be considered when selecting a suitable chromatography plate.

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 be understood before your stored compounds or samples are further analyzed.

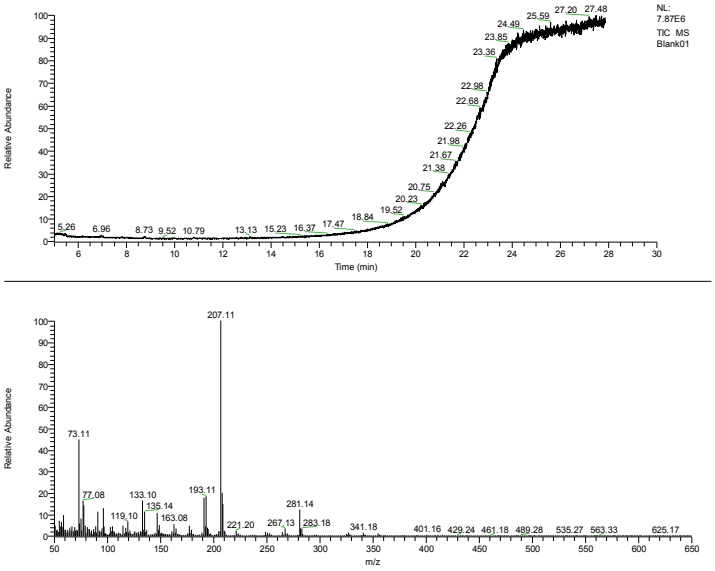
The introduction of a certified range of microtiter and deep well plates has been carried out to aid the user when utilizing MS and sensitive chromatographic analysis. These plates show minimal extraction with methanolic solutions as the raw material is a medical grade polypropylene with good solvent compatibility and minimal additives.

WebSeal plates (deep well plates with round and square wells with 1 and 2 mL volume) and PTFE covered silicone mats were used to produce the results in Figures 1 and 2. A number of plates with low levels of additive extracts have been chromatographically assessed and have been found to give low background suitable for routine HPLC applications.

This study also shows that external contamination of plates should be avoided. The use of silicone fluids may give a characteristic profile in total ion current (TIC) mode due to the presence of Si (n) oligomers.

Finally, some plates exhibit significant contamination from plastic extraction of mold release and lubrication products and should not be used due to potential interference and suppression in this mode.

Blank



WebSeal Test Plate

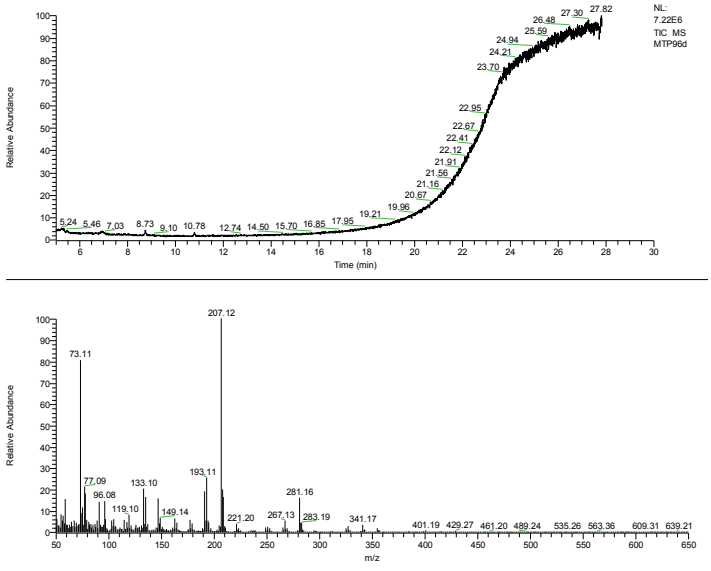


Figure 1: Comparison of blank methanol sample injection and a test plate with minimal extraction components.

Figure 2: Certification traces used for characterization of low background polypropylene plates. No significant additional peaks are seen in the sampled plate, compared to an injection blank. This has a low background suitable for MS analysis with minimal suppression from potential extraction compounds.

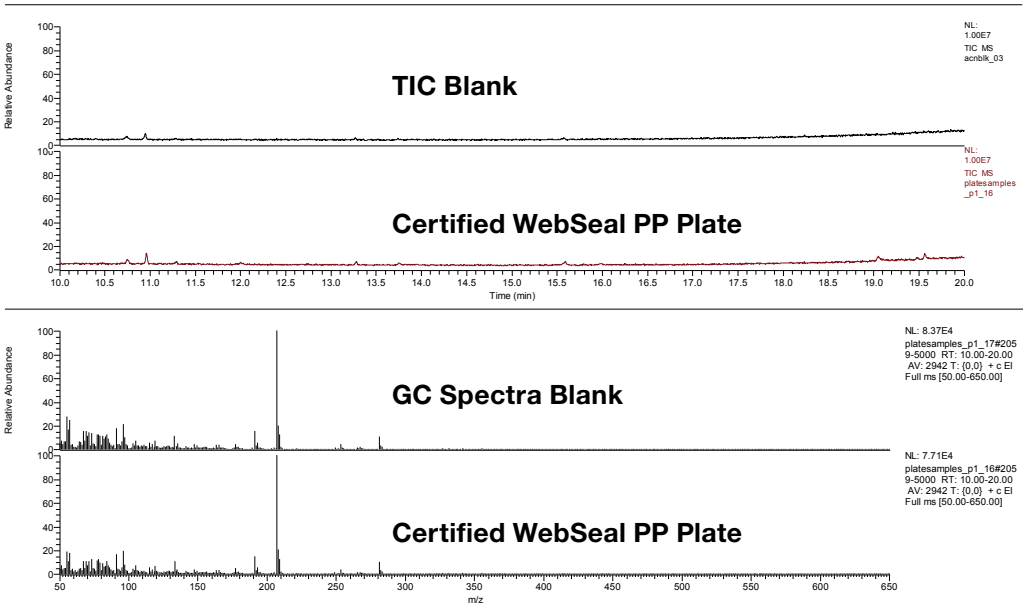


Figure 3: Example of a chromatography tested plate with low levels of extractants which may be seen at low levels.

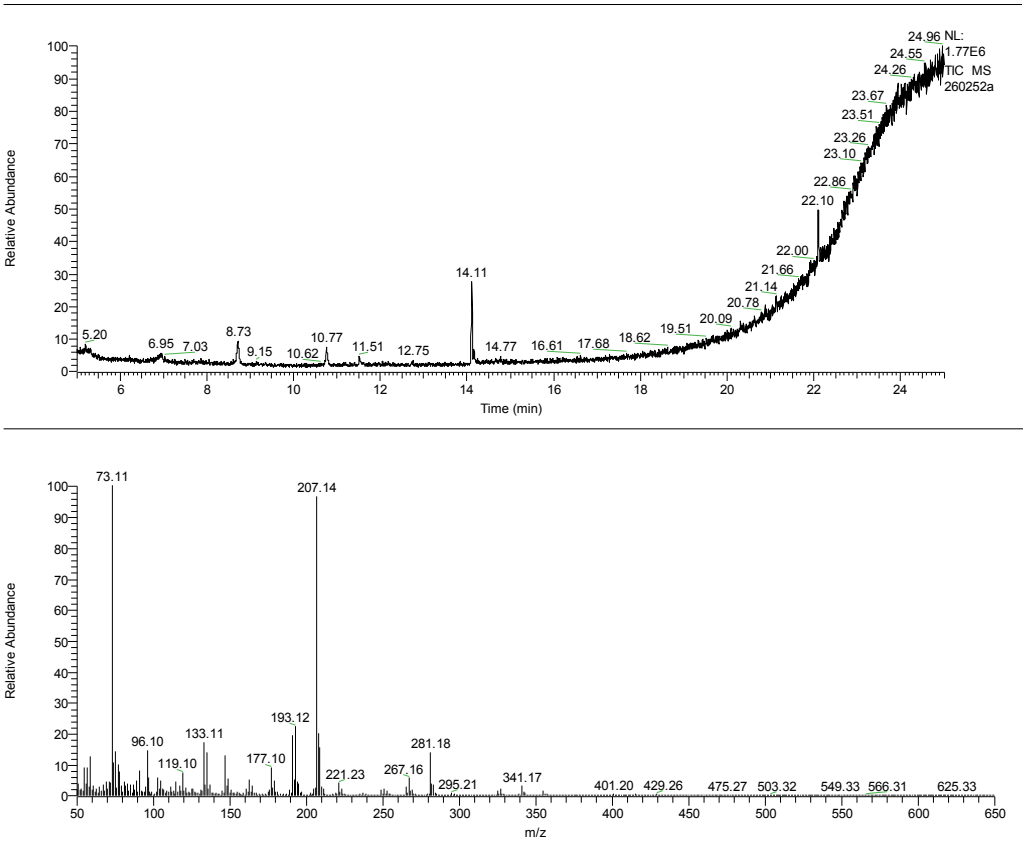


Figure 4: Match of extractant example from a lower quality polypropylene plate. Match was 95.71% when reprocessed. Compound is a component of a Phenolic Primary Antioxidant for processing and long-term thermal stabilization of polyolefins.

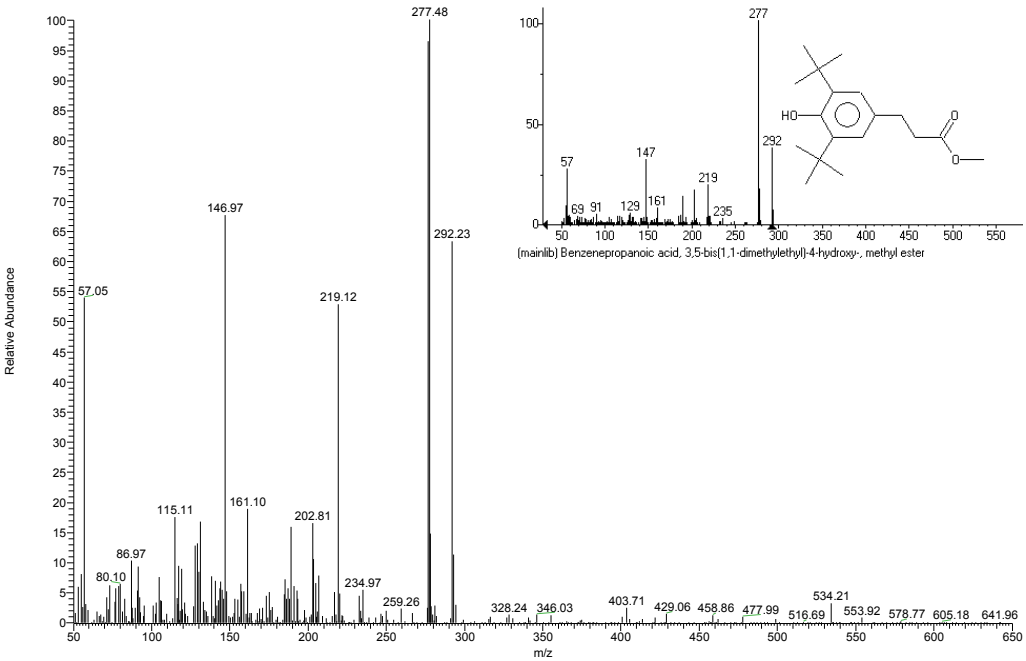


Figure 5: Example of methanol extraction from a poor quality polypropylene plate with antioxidant extract showing at 13.31 min with high extraction background from C16–C18 fatty acids.

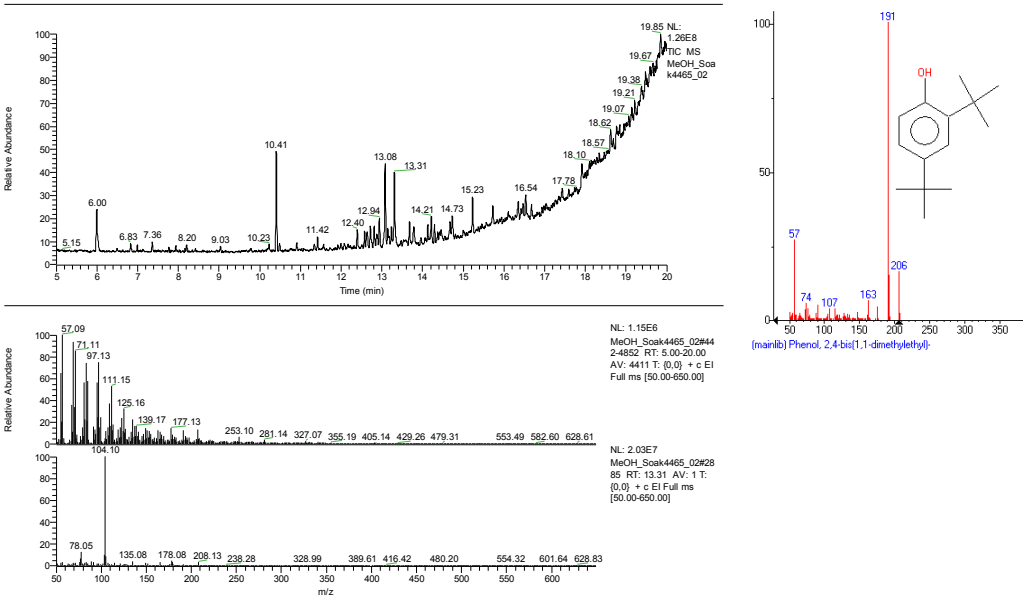


Figure 6: Example of external contamination with silicone fluid from processing of plates. This can also be observed if unprotected silicone sealing mats have been exposed to solvents during storage on the plates.

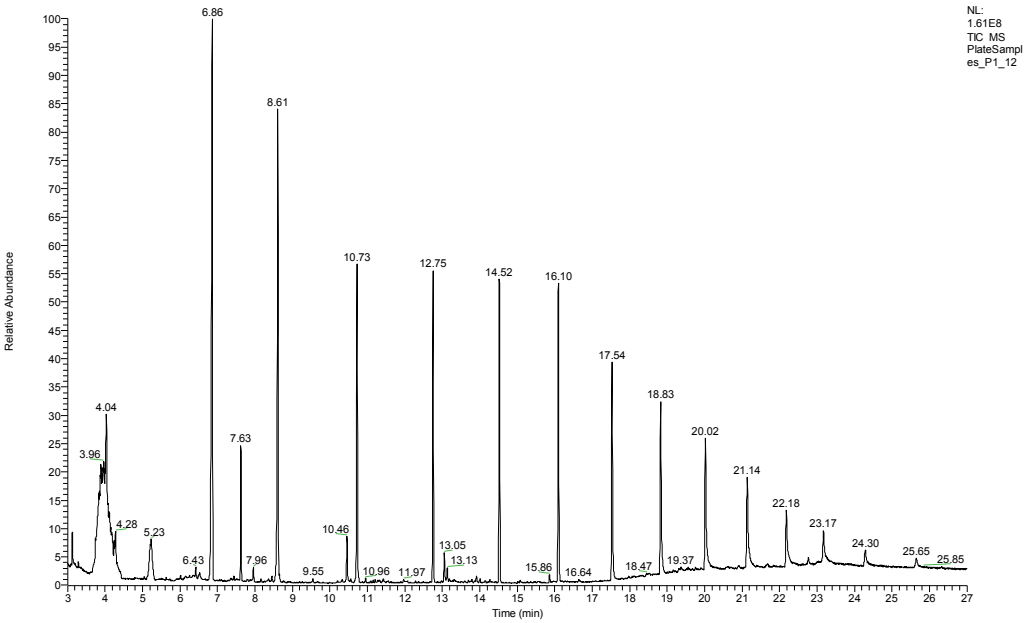
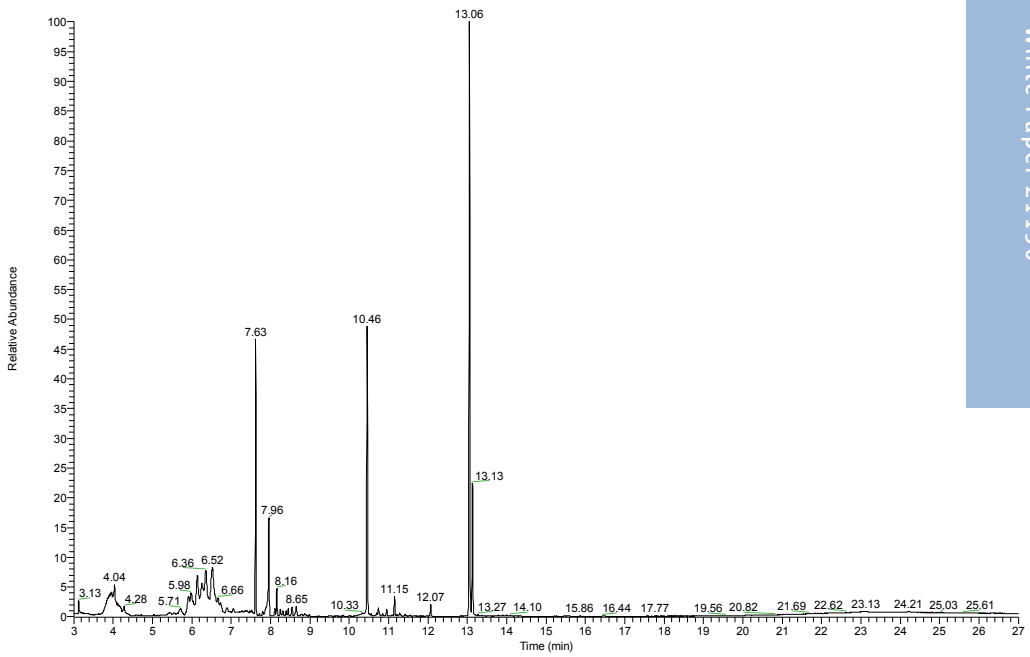


Figure 7: Contaminated plate extraction with example of multiple component contamination.



Products Used in This Paper

WebSeal Well Plates, Non Coated, Non Sterile, Chromatography Certified

Description	Well Format	Total Volume (µL)	Working Volume Range µL/Well	Catalog Number	Pack of
96-Well Microplate, Round Well	U-Shape, 7 mm Diameter	1000	50–900	60180-P211	5
	U-Shape, 7 mm Diameter	1000	50–900	60180-P201	50
96-Well Microplate, Square Well	V-Shape, 8 mm Diameter	2000	50–1900	60180-P212	5
	V-Shape, 8 mm Diameter	2000	50–1900	60180-P202	50

WebSeal Mats, Non Sterile

Description	Color	Material	Well Design	Pre-slit	Catalog Number	Pack of
WebSeal Mat	Blue	Silicone/PTFE	96 Round Well – Flat Base, Step, 7 mm Diameter	No	60180-M111	5
	Blue	Silicone/PTFE	96 Round Well – Flat Base, Step, 7 mm Diameter	Yes	60180-M112	5
	Blue	Silicone/PTFE	96 Square Well – Flat Base, Step, 8 mm Diameter, Large Penetration Area	No	60180-M120	5
	Blue	Silicone/PTFE	96 Square Well – Flat Base, Step, 8 mm Diameter, Large Penetration Area	Yes	60180-M122	5

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