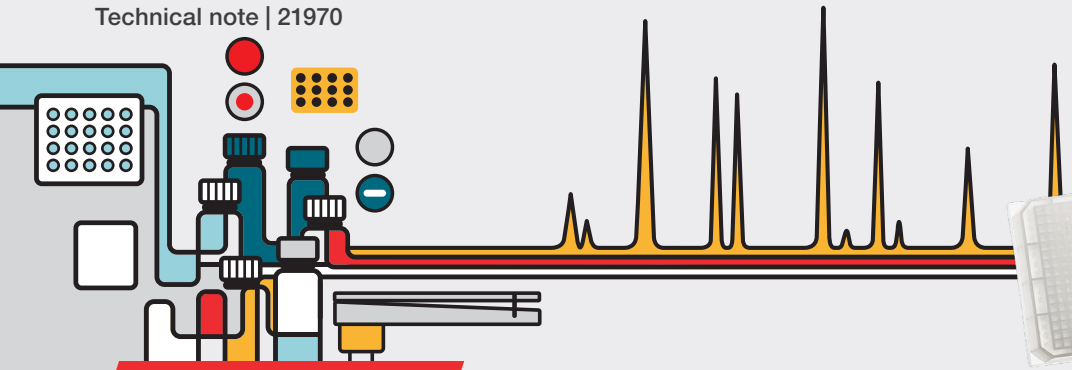
Technical note | 21970



Plastic well plates

Can a plastic well plate replace chromatography autosampler glass vials? The simple answer is: yes!

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Keywords

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Introduction

Glass-coated polypropylene (PP) plates have been on the market for decades now. They are a solution for customers who cannot use pure plastic plates due to either solvent compatibility issues or a loss of product due to adsorption on a pure plastic wall. Adsorption on cavity walls is the number one reason for poor reproducibility in bioanalysis. As the wall polarity in a glass-coated plate is inverted from non-polar to a polar surface, it has to be concluded that the risk of adsorption is significantly reduced.

The new 2nd generation plate+ products are made of high-quality PP, designed with state-of-the-art technology to deliver higher density coating (thickness of 200Å), improved solvent stability, and better reproducibility, especially for highly on plastic walls adsorbing biomolecules. The Thermo Scientific™ Webseal™ Plate+ 2nd products provide all usage benefits of a plastic well plate (robotic handling, multi pipette usage, etc.) with features of a glass surface at more attractive prices than a pure glass plate.

Background

How can it be beneficial to think about using a well plate for chromatographic applications when glass vials have been the preferred solution in liquid chromatography (LC) and gas chromatography (GC) autosampler for at least four decades? Glass vials are single-use, single-operation sample handling containers. The filling and closing operation is always a single vessel activity. On the contrary, a well plate is a high-throughput, multi-cavity container, where the industry offers many helpful tools to automate the filling, handling and sealing of multiple cavities. In fact, sample preparation and titration robots are fully designed to use and handle well plates as a preference. Moreover, here we face a bottleneck in modern chromatography workflow: biological samples are frequently prepared and handled

in well plates as standard procedure and as the standard container. But, until now in LC and GC chromatography, well plates are only rarely used. Here are reasons why some applications require a glass vial:

A) Glass vials are used because in chromatography, organic solvents are used and therefore a solvent stable and inert sample handling container is required.

B) Vials are sealed tightly and reliably with caps and septa, which also provide an evaporation-safe environment.

C) The septa needs to be piercable for autosampler needle operation and should re-seal after penetration. Therefore, most septa contain an inert layer - typically polytetrafluoroethylene (PTFE) on the rubber part of the septum. The rubber is required for the compression, and seals and re-seals to hold pressure.

For applications and workflows in bioanalysis and preparation of biological samples where plates are in regular use, plastic well plates are typically used (e.g. with 96 or 384 cavities). The plastics can be polystyrene, polyacrylics or polypropylene (PP). For chromatography applications, only PP plates can be used because other plastics have poor stability against organic solvents. In addition, PP is most stable against polar, with water miscible solvents. As soon as the solvent becomes more non-polar or when aggressive reagents are added, PP is no longer usable due to material destruction. Therefore, glass-coated well plates are the perfect option and solution for the above mentioned cases. They provide a like-working-with-a-glass-vial environment.

A similar issue comes up with sealing options widely used in bioanalysis and preparation of biological samples: either the plates are left open as water does not evaporate rapidly, or adhesive sealing foils made from aluminum or plastic are used. With 100% water samples, this is not an issue, but volatile organic solvents cannot be left without a seal. As many of these seals contain adhesives, compromising the samples integrity is a major risk. The glue dissolves and it drops into the cavity and contaminates the sample. The only effective solution here is to use silicone mats with or without PTFE treatment.

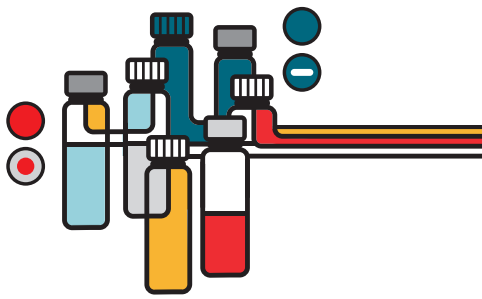
Method

The following protocols were used to create a comparison of non-coated polypropylene plates to glass-coated polypropylene plates, by analyzing extractables with gas chromatography-mass spectrometry (GC-MS).

Procedure

A sample of fresh, unused well plates was taken from multiple production batches and several wells were tested for leachables. Several wells (15) of the test well plate including the corner and center wells are incubated with an appropriate volume of HPLC grade Methanol (MeOH) for 24 hours. The MeOH contained Nonadecane (C19) internal standard at the indicated concentrations (0.25 ppm, 0.5 ppm, or 1 ppm). After 24 hours, extracts from selected wells were pooled, mixed, and an aliquot transferred to a suitable glass chromatography vial and analyzed by GC-MS. For comparison, blank vials containing only MeOH and the C19 internal standard were also prepared.

All chromatograms are compared to a blank run of C19 internal standard in methanol. The internal standard of C19 was used to evaluate the peak heights of the resultant chromatogram. Sample chromatograms were background compensated by subtracting selected blank injections (chromatography software feature).



Instrumentation method

GC-MS

Column:	Type 5 column with guard column, 17 m × 250 µm × 0.25 µm
Carrier gas:	Helium
Flow rate:	1 mL/min
Oven program:	(three methods) 10 minute method: 70 °C, hold for 2 min; 40 °C/min to 175 °C, hold for 0 min; 35 °C/min to 300 °C, hold for 0 min; 30 °C/min to 320 °C, hold for 1.14 min 15 minute method: 70 °C, hold for 2 min; 25 °C/min to 320 °C, hold for 3 min 25 minute method: 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:	260 °C
Inlet configuration:	Splitless, 1 min
Injection volume:	(depends on instrument and method, injections usually contain internal standard) 1 µL for 10 min, 15 min and 25 min method 0.2 µL for 10 min method 0.5 µL for 15 min method
MS transfer line:	260-280 °C, depending on method.
MS ion source:	230 °C
MS quad:	150 °C
MS detection:	Positive EI; Full scan *m/z* 33-410

The plates used for the experiment:

Type	Material	Made in	Figure	Cat. no.
Thermo Scientific™ WebSeal, 96-deep well, round wells	Polypropylene	EU	1	60180-P201
Thermo Scientific™ WebSeal™ Plate+, 96-deep well, round wells	Glass-coated PP	US	1	60180-P336
Thermo Scientific™ WebSeal, 96-deep well, square wells	Polypropylene	EU	2	60180-P135
Thermo Scientific™ WebSeal™ Plate+, 96-deep well, square wells	Glass-coated PP	US	2	60180-P338
96 microtiter, round wells	Polypropylene	EU	3	Competitor P
Thermo Scientific™ WebSeal™ Plate+, 96 microtiter, round wells	Glass-coated PP	US	3	60180-P334
96 mid-height, round wells	Polypropylene	CN	4	Competitor G
Thermo Scientific™ WebSeal™ Plate+, 96 mid-height, round wells	Glass-coated PP	US	4	60180-P343
Thermo Scientific™ WebSeal, 96 mid-height, round wells	Polypropylene	EU	5	60180-P154
Thermo Scientific™ WebSeal™ Plate+, 96 mid-height, round wells	Glass-coated PP	US	5	60180-P350
Thermo Scientific™ WebSeal™ Plate+, 96 microtiter, round wells	Glass-coated PP	US	6	60180-P334
Thermo Scientific™ WebSeal™ Plate+, 96 microtiter, round wells	Glass-coated PP	US	7	60180-P334

The glass vial used for the experiment:

Type	Material	Made in	Figure	Cat. no.
Thermo Scientific™ SureSTART™ GOLD-Grade Inert Glass Vial, 2 mL	Clear glass	EU	Reference glass vial	6PSV9-1PG

Comparison

Figure 1 shows a comparison of a certified non-coated pure polypropylene Thermo Scientific™ Webseal™ 96-Well Plate (P/N 60180-P201) with a blank chromatogram from a Thermo Scientific™ SureSTART 2 mL glass vials with GOLD-grade glass and *SureStop* technology (P/N **6PSV9-1PG**) and the result from the Thermo Scientific™ Webseal™ Plate+ 2nd generation (P/N **60180-P336**). It is obvious that the coating "removes" some spikes from the PP surface or a non-coated plate and shows an identical result to a pure glass vial. Nevertheless, the certified Webseal polypropylene plate is not a bad choice, even after 24 hours with MeOH. The major difference is the inversion of polarity of the surface: the PP plate is non-polar, the coated plate+ is polar. For storage or long-time applications with solvents, the glass-coated plate+ is highly recommended.

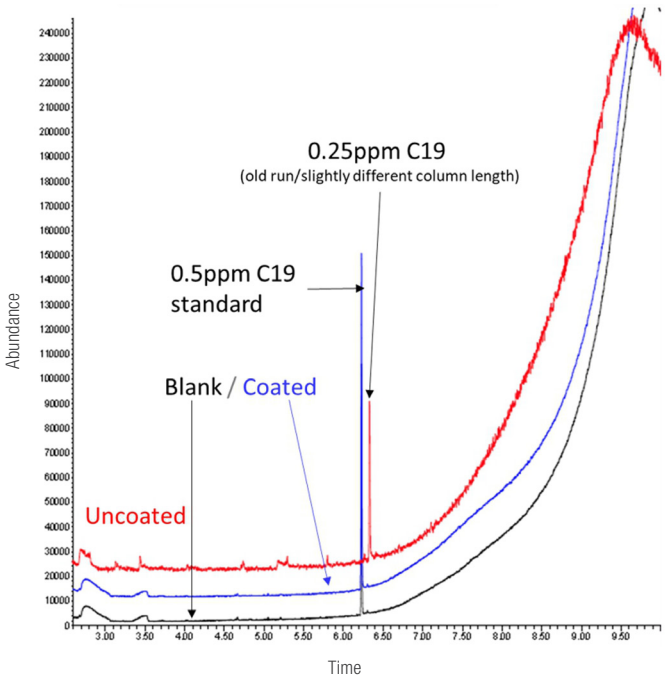


Figure 1.

When using a Thermo Scientific™ WebSeal 96-Well Non-Coated Plastic Microplate (P/N 60180-P135), the picture appears slightly different. In Figure 2, the non-coated plate shows a higher - but still acceptable - degree of leachables under Methanol treatment compared to the certified plate in Figure 1. Here, as well, the glass-coated plate of the same type and size is free of contaminants.

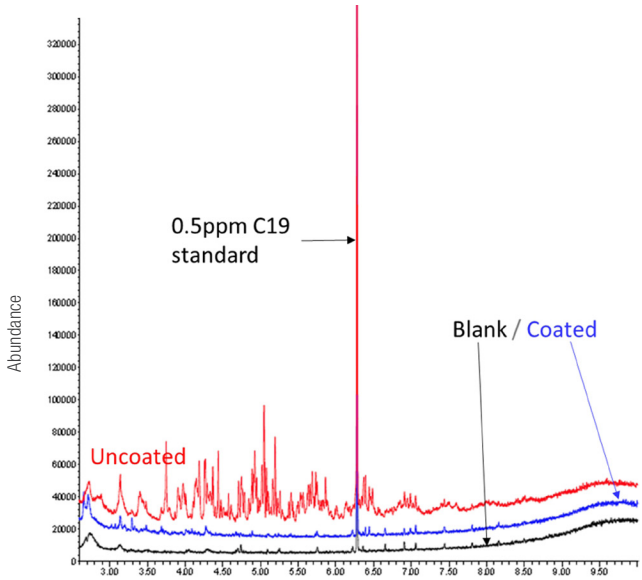


Figure 2.

The same analyses with competitor plates P and G (Figures 3 and 4) show, why not every standard PP well plate can be used for chromatographic applications with a significant content of organic solvents. Non-treated PP plates show extractables. Both Figures 3 and 4 are separated into two parts: low- and high-resolution. The GC-MS indicates contaminants, appear after MeOH incubation over 24 hours. They can be identified as Methyesters of Hexa- and Octadecanoic acid and Glycerylesters of Hexa- and Octadecanoic acids. It is believed that these are mold-release agents, presumably added to the raw resin used in molding, but also possibly applied to the mold during processing and can be found in many plates on the market. Here, as well, a coated plate+ of the same dimension shows virtually no sign of these mold-releasing reagents. In the cases shown here, the small peaks, which appear at regular intervals in the baselines, are caused typically from the silicone sealing mat. When an organic solvent touches the silicone, this silicone "ladder" appears (= extraction of soluble mono-, di-, tri-, ……. mers, which are not 100% polymerized and therefore accessible for to any organic solvent).

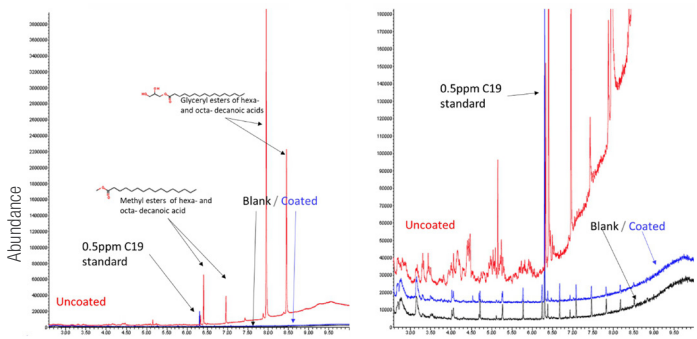


Figure 3.

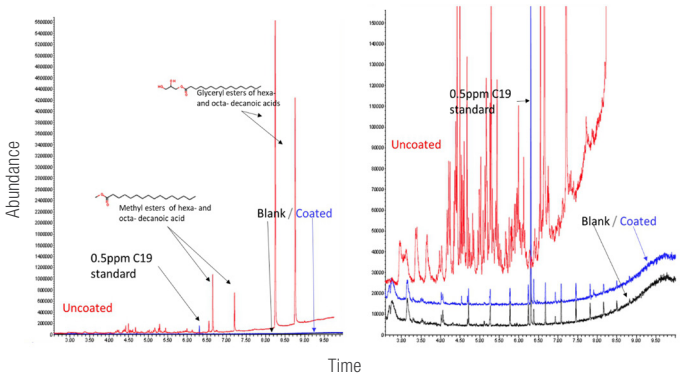


Figure 4.

How good, accurate and reproducible is the glass coating across cavities and from plate-to-plate or batch-to-batch?

Figures 5a and 5b provide an overview of what was tested in terms of cavity statistics and the results. Here the mid-height plate, P/N **60180-P350**, was tested and 5 cavity pairs of three were collected and combined in order to give a representative complete picture. Figure 5b as well shows the extractable profile of the non-coated basic plate P/N 60180-P154 (brown trace) used for the coating. Black and purple traces are blanks, all other remaining traces are from the indicated wells (see Figure 5a) from the coated plate.

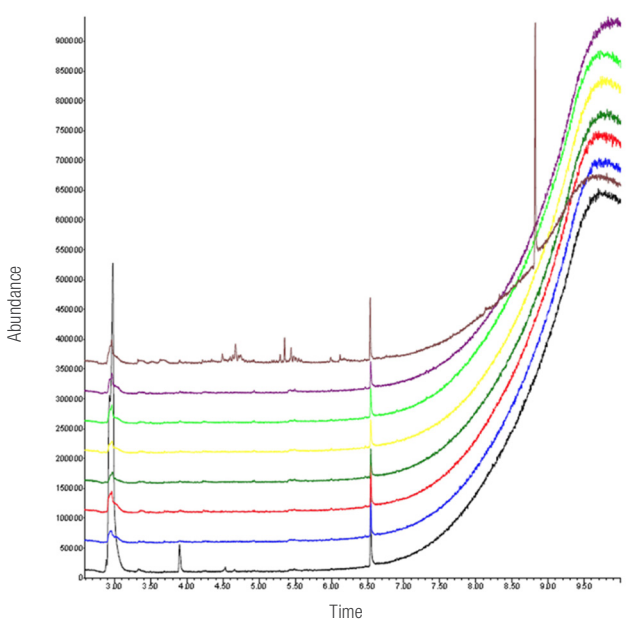
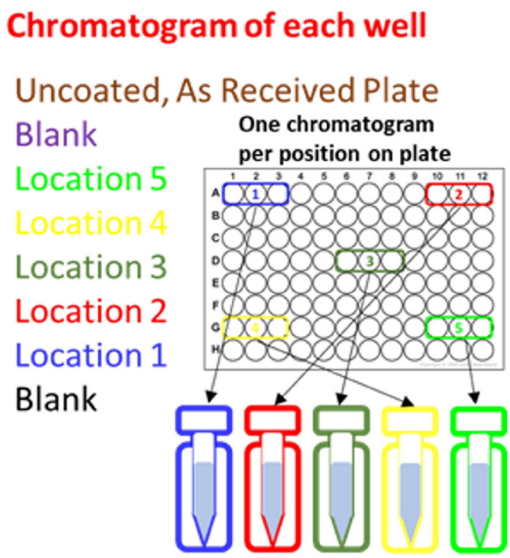
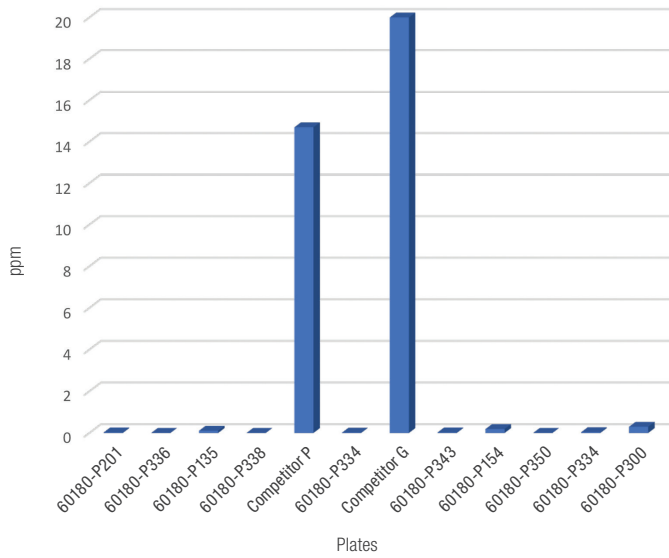


Figure 5b.

For a better overview of how significant the level of leachables/ extractables can be with MeOH for 24 hours in the plate, Graph 1 shows a comparison of all tested and investigated products with their contaminant levels
in ppm.



Graph 1. Contamination level with MeOH [ppm]



Results shown so far have been created with MeOH as polar organic solvent. What happens when a hydrophobic, more aggressive solvent like ethyl acetate is used, is shown in Figure 6 with plate P/N 60180-P334. It is seen that a first breakthrough and increase of extractables starts after about 8 hours at Trial 2, with increasing impact after 24 hours. As a consequence, the >200 nm thick glass coating seems not to be sufficient enough and is not recommended for an application with high (100%!) non-polar solvent over a longer period. This breakthrough effect is not visible in every plate and every cavity; trial plates 1 and 3 do not show this effect.

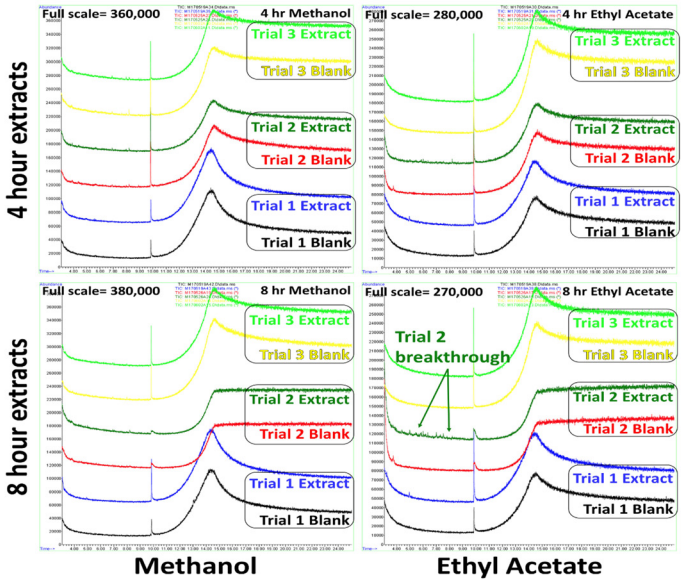


Figure 6.

The proof, that the new 2nd generation plate+ with its state of the art coating technology is a real improvement is visible in Figure 7a. On the left, the MeOH extractables of a non-coated plate are compared with the new coated plate P/N 60180-P334. On the right, the non-coated plate was not investigated, but the level of extractables after 4, 8 and 24 hours of the 1st generation plate+ P/N 60180-P300 indicate, that the coating show some breakthrough even with a polar solvent like MeOH. More obvious this effect can be seen using a non-polar solvent like ethyl acetate. Figure 7b shows the extractable profile of the new P/N 60180-P334 compared to the "old" P/N 60180-P300.

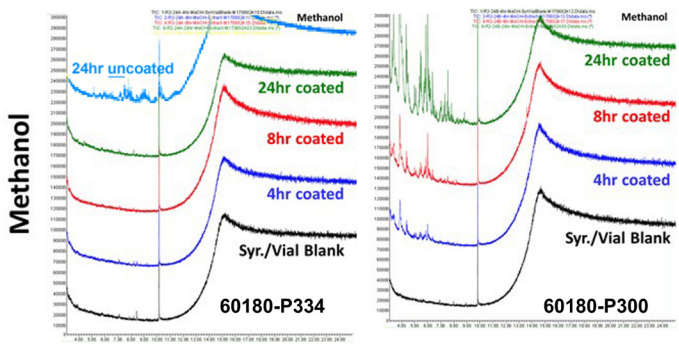


Figure 7a.

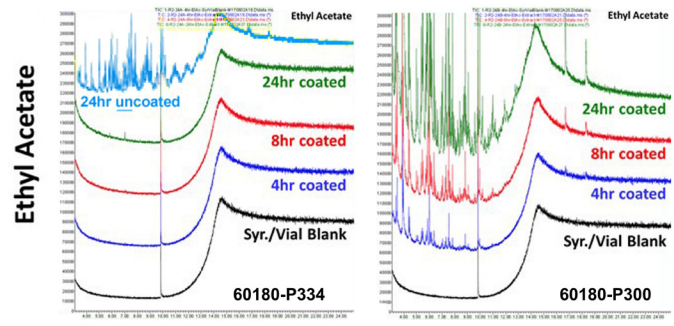


Figure 7b.

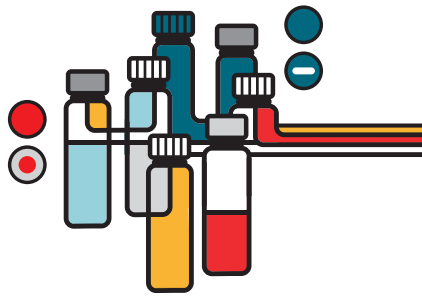
Results/conclusion

Using a pure plastic plate for chromatographic applications can be either a challenge or simply not possible due to solvent compatibility. The new 2nd generation plate+ solves this issue for polar solvents, even after extended incubation. The 2nd generation Plate+ coating significantly reduces or eliminates contamination of samples by plastic leachables. In addition, the glass coating inverts the polarity of the non-treated plastic to reduce non-specific adsorption in bio-analysis.

The results cited here for the stability of the newly-formulated Plate+ indicates that these plates are outstanding candidates for analysis requiring water-miscible organic solvents for LC, LC-MS or ultra high performance liquid chromatography (UHPLC) in any required concentration. A limited timeframe of about 8 hours may be achieved for non water-miscible solvents that have some polar character and water solubility (e.g., ethyl acetate). However, usage of aggressive, highly nonpolar solvents (non water-miscible) has not been tested and therefore can not be recommended for any period of time.

Plate+ products are unique in the market and offer users of LC and GC autosamplers an option for high-throughput applications, where it is not convenient, to use single vials. These plates offer the full benefit of well plate usage including automation, filling plates parallel (multi-pipettes), reducing lab space requirements, more efficient long-term storage and expanding autosampler capacity. Normal ANSI SLAS 4-2004 (R2012) format autosampler racks can only handle 54 glass vials and now can be replaced by 96- or even 384-cavity plates.

Additionally, the plate+ product solves a significant workflow bottleneck in the pharmaceutical and biopharmaceutical industries: samples prepared on titration and sample preparation robots can directly been transferred into the chromatography autosampler without re-pipetting or transfer in alternative sample handling container.



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