



## To the limits of analytical detection and beyond

### NANOPARTICLES

DETECTING  
INVISIBLE  
POLLUTANTS

### STATINS

OBTAINING  
MEDICINE FROM  
NATURE

### DRUGS

BATTLING  
ILLEGAL  
COMPOUNDS

### HORMONE-MIMICS

ENDOCRINE  
DISRUPTION  
AND HEALTH

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## The solution behind great science

To the limits  
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# Introduction

Thousands of scientists around the world are making use of cutting edge technologies every day in order to push the boundaries of what we thought possible, furthering our understanding of the world around us. This dedicated pursuit of knowledge rests upon the shoulders of advances in the development of analytical instrumentation, specifically built to allow us to probe ever deeper into the processes governing the fundamental aspects of biological, chemical and physical systems.

Whether one is trying to diagnose disease or understand the mechanisms behind global warming, the ability to make accurate, quantitative measurements is essential for identifying, classifying and monitoring the important factors driving any given process. To do this successfully requires the use of highly sensitive tools, rigorous detection processes and precisely calibrated equipment. The use of advanced systems in the fields of chromatography, microscopy and spectroscopy are enabling scientist to explore and quantify the realms of the infinitesimal. From patients to populations, ensuring a positive outcome can depend largely on the insights provided by advanced analytical detection.

When examining the exceedingly small, it becomes increasingly relevant to use the highest quality reagents available. It's important to be sure that when conducting fine scale analysis that what you are detecting is the sample of interest, rather than a piece of unwanted contamination! For this reason, pure water is an unsung hero of every experiment, especially those working at the limits of analytical detection. As experts in pure water for use in the laboratory, we're proud to help fuel some of the world's most exciting science.



Here we take a closer look at some of the current research being conducted at the limits of detection. First, we explore how the detection of nano-sized particles in the environment could help improve human health, before turning our attention to the extraction of cholesterol-lowering statins from wood samples. Next, we look at how the US Drug Enforcement Administration is targeting new 'designer drugs' using advanced analytical systems. Finally, we report on the latest research into the effects of common plastic polymers on human disease (the likes of which could be costing the European Union alone up to €31 billion a year!).

These research projects are helping to further our understanding of the natural world, improve medicine and protect us from the unseen dangers all around us. We hope you'll enjoy learning about them as much as we did! You may even be carrying out similar, cutting-edge work in your own labs – if so we'd love to hear from you. Get in touch today and maybe we'll be able to feature your work in the next edition of 'The Solution Behind Great Science'.

Best regards,

**The ELGA Editorial Team**



# Monitoring the invisible: Nanoparticles in the environment

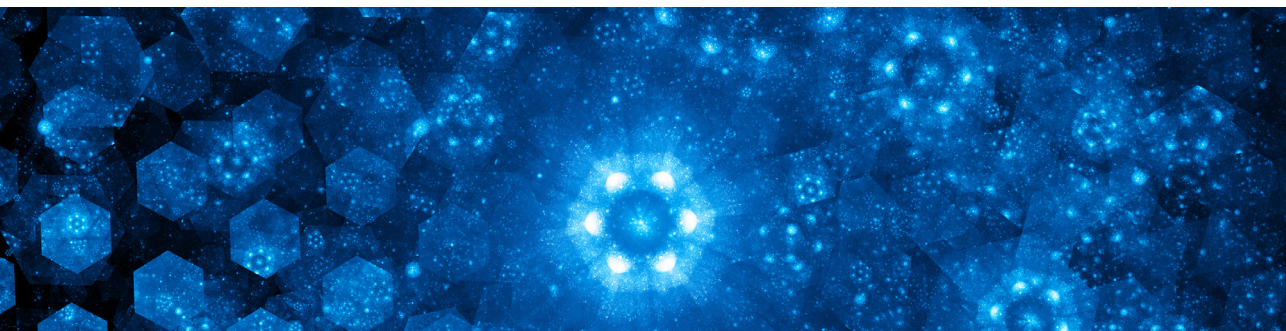
Nanotechnology is now commonplace across industry, and engineered nanoparticle (ENPs) are being used in the development of a wide range of products that most of us use on a daily basis, from electronic devices to sun cream lotions. From a financial standpoint, the world nanotechnology market is big business, and is expected to exceed \$30 billion by 2015. However, with this rise in use comes the concern over the potential effects these invisible particles may be having on the environment and human health. For example, ENPs that become airborne can be inhaled by people leading to respiratory effects<sup>1</sup>, whereas in the aquatic environment ENP exposure can lead to tissue accumulation and oxidative stress<sup>2</sup>.

ENPs themselves come in a range of shapes, typically have one dimension less than 100 nanometers (nm) in length and can be present in natural water systems at concentration ranges as low as nanograms (ng) per liter (i.e. parts per trillion). Accurately detecting and quantifying such minute amounts is a challenging task, requiring high grade reagents and advanced equipment. However,

such research is vital if we are to better understand and predict the toxicity of ENPs. Further complicating the matter, the behavior (and thus toxicity) of ENPs can be significantly influenced by environmental factors such as pH and salinity, as well as the presence of divalent ions and natural organic matter. As such, knowledge of these interactions is essential for accurately predicting the impact of ENPs on human health.

Recent work conducted at the National University of Singapore tackled this issue head on in an attempt to better understand how a combination of environmental factors can influence the fate, behavior and exposure risks of zinc oxide nanoparticles (ZnO NPs)<sup>3</sup>.

A large proportion of ecotoxicological studies focus on understanding the effects of a single, isolated variable. While this makes data analysis relatively straightforward, it fails to reflect the dynamic interactions that actually occur in nature. Based on this, the researchers examined the combined effects of environmentally relevant conditions (organic acid type and concentration,



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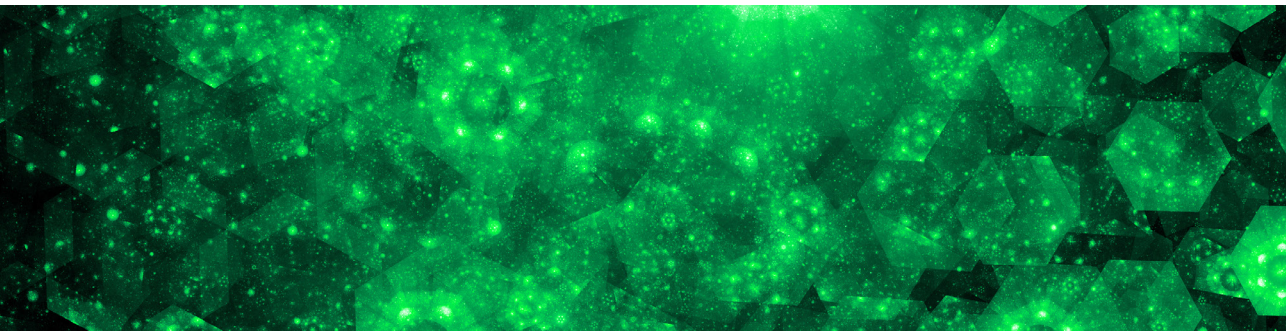
NP concentration, pH, electrolyte type and concentration, and temperature) and used powerful multiparametric methods (an orthogonal array design) to assess the relevant contribution of each factor. The research was also the first of its kind to assess the significance and contribution of temperature to ZnO NP dissolution under these combinatorial conditions.

Making use of a dynamic light scattering (DLS) analyzer, the team set out to explore this complex, co-varying system to determine how ZnO NPs might really behave in the natural aqueous environment. Their highly interesting results showed that the ENPs behave differently when interacting with different combinations of factors, further highlighting the relevance of their improved approach for modelling ENP behavior in environmental systems.

Organic acid concentration and pH were found to be the most significant influencers of ZnO NP aggregation and dissolution respectively. It has been previously shown that the aggregation of zinc compounds to form complexes greatly lowers the concentration of available ions,

thus reducing their toxicity<sup>4</sup>, while rapid dissolution will increase the availability of free ions, increasing the levels of toxicity<sup>5</sup>. There was also a very clear temperature-induced effect on aggregation, something that may have increasing importance if Earth's surface water temperatures continue to rise.

By building robust models utilizing multiparametric methods that incorporate several environmentally relevant factors, we are able to predict the fate and behavior of not just ZnO NPs, but also the potential environmental impact of other contaminants. A combinatorial model of toxicity is a much better reflection of the conditions observed in nature. By conducting such studies, and continuing to build functional models that better mimic the physicochemical properties and behaviors of ENPs in the environment, we are constructing a powerful tool for accurately predicting exposure risks. This would ultimately allow governments and regulators to develop more robust policies and practices for better managing the presence of ENPs within the environment.



# Searching the woodland for statins

Statins are a class of drug used in the control of cholesterol, which act by inhibiting the enzyme HMG-CoA reductase; today an estimated 30 million people worldwide are taking statins to reduce the risks associated with cardiovascular disease<sup>6</sup>. Naturally occurring molecules, statins have a long history and were first identified in 1973 following their isolation from the fermentation fluid of the mould *Penicillium cintrinum* (for detailed reviews see Tobert, 2003 and Endo, 2010)<sup>6,7</sup>. This early statin was named compactin (later changed to mevastatin) and its cholesterol-lowering properties sparked a great deal of interest: next came lovastatin, produced from *Aspergillus terreus* mold fermentation.

Naturally occurring statins can also be found in human foods such as oyster mushrooms and red yeast rice, while species of fungi from the genus *Pleurotus* produce statins as a by-product, which is formed by breaking down lignocellulose as they feed on dead wood<sup>8</sup>. Today there are seven additional statins used in modern medicine, some of which have been created synthetically based on existing natural molecules.

A team of researchers from the University of Helsinki are investigating naturally occurring statins and recently set out to identify them in wood samples treated with *P. ostreatus* via gas chromatography–mass spectrometry (GC-MS) using total ion monitoring (TIC) and multiple reactions monitoring (MRM)<sup>9</sup>. Samples of bark, phloem and core were powdered and treated with microbes for fermentation before final water extraction. Making use of ultrapure water, the team were able to separate out the different forms of statins from the wood sample, precisely quantifying the relative amounts present down to ranges within 50–1000 parts per billion (ppb).





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The team showed that, while the core samples only contained pravastatin at very low levels of detection, the pine phloem extracts included four types of statins, as well as two other statin esters.

The study is not only the first of its kind to use GC-MS methods for use with biorefinery samples (pine bark, phloem and core), but also provides valuable insight into the optimization of wood sample analysis for the generation of biocompounds such as statins.

The use of statins over the years has been met with a range of responses; while some forms have been recommended for widespread use in controlling heart disease, others have been reported to cause unwanted side effects. For this reason, the search for novel statins that offer medical benefits without any of the drawbacks goes on.

Advances in technology such as those described above are driving this research. Other active areas of interest include the use of three-dimensional quantitative structural activity relationship (3D QSAR) analysis to identify new compounds with statin-like mechanisms of action, while other research teams are exploring the possibility that existing statins might find use in other areas, such as dementia and even cancer.

Given the expanding applications of statins, combined with the fact that cardiovascular disease is still one of the biggest killers in the developed world, we can expect to see even more research aimed at improving the identification, isolation and medical optimization of these important molecules.





# Chromatography and the clamp down on illegal drugs

The term 'designer drug' was coined by the forensic community back in the 1980s to denote non-controlled analogues of currently controlled substances. Since then there has been an explosion in their use, accompanied by a wave of reported side-effects and even deaths.


By 2012, the US had placed 26 designer drugs within Schedule I of the US Controlled Substances Act. Following this, and in an attempt to clamp down on these drugs, there has been a growing need to develop methods that can be used to quickly identify 'designer drugs'. Failure to do so allows manufacturers to generate structurally similar versions of illegal drugs and then market them as legal alternatives.

To meet this need, forensic labs across the globe are in a constant battle with drug developers, working hard to develop better analytical methods to identify unknown substances.

The primary screening of suspected drug samples is typically conducted using GC-MS and GC with a flame ionization detector (GC-FID), but these techniques can fall short on account of the chemical nature of certain classes of chemical. For example, the thermal instability of some synthetic cannabinoids/cannabimimetics can result in their breakdown during the GC process, while the extraction of phenethylamine and cathinone derivatives, as is required to generate reliable chromatographic data, is a time-consuming process. Fortunately, HPLC-based methods do not suffer from many of these limitations; HPLC-photo-diode array (HPLC-PDA) analysis has previously been employed to generate libraries of designer drug signatures, which can then be used to detect similar structures in test samples<sup>10</sup>. While this is an improved approach, there still exists a need for an efficient, versatile, primary screening procedure that extends the usefulness of traditional GC-MS, GC-FID and HPLC-PDA analysis.

*“there has been a growing need to develop methods that can be used to quickly identify ‘designer drugs’”*





To this end, researchers working in the US Drug Enforcement Administration's Special Testing and Research Lab are investigating the use of a dual detection system – ultra HPLC-PDA combined with ultraviolet MS (UHPLC-PDA/UV-MS) – as a powerful preliminary screening tool for detecting a broad spectrum of emerging drugs<sup>11</sup>. To ensure sampling integrity and avoid unwanted contamination of the samples, the research team use high purity water from an ELGA LabWater PURELAB Ultra Mk2 system.

The results generated to date are very promising. The use of the dual detection system is able to provide not only information on the compound's molecular weight, but can also successfully differentiate drugs by category or even subcategory.

Also, the addition of UV spectra analysis allows for the easy differentiation of positional isomers of certain drug categories; even a shift in structure isn't enough to evade detection from the method, allowing it to be used to detect positional isomers of both phenethylamine and cathinone derivatives. Rapid screening technologies such as UHPLC-PDA/UV-MS allow for faster, more accurate identification of designer drugs and their related analogues, enabling drug enforcement agencies to better control their use and traffic. We'll be interested to see how these methods continue to develop in the future, supporting the world's governments in the fight against illegal drugs.



# From plastics to patients: Understanding the impact of EDCs

Bisphenol A (BPA) has been around since the 1950s and is used to make an array of plastics and epoxy resins. However, BPA is also classed as an endocrine disrupting compound (EDC). EDCs are chemicals that interfere with the endocrine system, typically by mimicking the action of various hormones. Their effects are wide ranging, varying from problems in cognitive development and the triggering of cancer, to sexual developmental complications such as the masculinization of females (imposex in dog whelks, for example) or the feminization of males.

Even very low levels of EDCs can induce sub-lethal, chronic effects, making their toxicological assessment especially difficult; due to practical limitations, a large percentage of safety testing relies upon using doses that are typically lethal, and thus chronic, sub-lethal effects are often overlooked. For those looking to investigate these sub-lethal effects, the main challenge is to devise a protocol capable of accurately determining the presence and effect of EDCs at very low concentrations.

Given its common use in food packaging, it is unsurprising that the estrogenic activity of BPA has raised concerns about its potential impact on humans. Being fully absorbed within the digestive tract following consumption of contaminated food, BPA is quickly metabolized to BPA  $\beta$ -D-glucuronide (BPA-gluc), which is then rapidly excreted via the urine. This presents an excellent means of assessing potential exposure. However, the levels of BPA-gluc in urine are often very low, rendering accurate quantification somewhat challenging.

In an effort to accurately determine these minute concentrations, researchers from Mersin University (Turkey) have developed a highly accurate, sensitive and selective method for detecting low levels of BPA using liquid chromatography–tandem mass spectrometry (LC-MS/MS)<sup>12</sup>. Since the amount of BPA present is exceedingly low, it is essential to remove any potentially confounding factors by using ultrapure water for diluting the test samples and setting up the analysis.



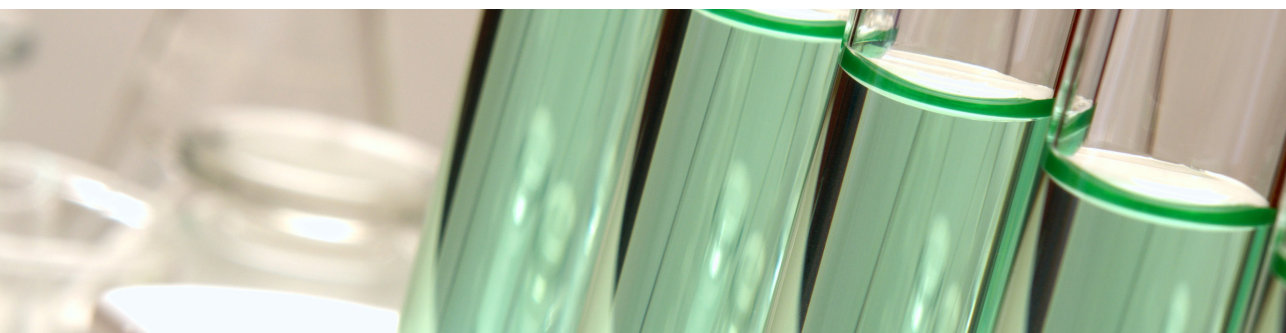


# *“the estrogenic activity of BPA has raised concerns about its potential impact on humans”*

The quantification method used by the researchers provided relative limits of detection for both BPA and BPA-gluc (at 0.03 ng/ml and 0.10 ng/ml respectively), with absolute limits of quantification being only marginally higher at 0.08 ng/ml and 0.33 ng/ml. Not only was the approach highly sensitive, but it was impressively consistent: the intra- and inter-day precision (percentage relative standard deviation, RSD) of the method was lower than 5%, well within the 20% maximum suggested by current FDA guidelines. It is hoped that this refined model will help drive the future development of HPLC-MS/MS analyses for quantifying both BPA and BPA-gluc levels in an attempt to save both time and money, without compromising on accuracy.

Improving the detection of EDCs such as BPA in human samples is very important. Exposure to food and everyday electronic, cosmetic and plastic products containing EDCs could be costing up to €31 billion per year in the EU alone, at least according to a recent 2014 report from the Health and Environment Alliance (HEAL)<sup>13</sup>.

New methods that can link even very low levels of EDCs to specific disease outcomes would improve our understanding of how these factors influence human health, helping to inform future policy on how we can reduce the impact of these widespread, but potentially hazardous, molecules.



# The solution behind great science

Ultra-sensitive technology is facilitating scientific investigation at hitherto unexplored levels of detail. However, the resolving power of much of this equipment is highly dependent upon the quality of the reagents used for the analysis; an inductively coupled plasma mass spectrometry (ICP-MS) study looking to identify particles or elements in the parts per trillion range will quickly be confounded by uncontrolled levels of organic content or other contaminants in solutions such as buffers or water used to dissolve/dilute samples. Because of this, the water used in equipment feeds, buffer preparation or sample dilution needs to be of the highest possible purity, in order to avoid significantly affecting the outcome of an experiment.

Fortunately, clearly defined categories of water have been devised so that investigators are able to select the right type of water for the work at hand (see **Tables 1 and 2**). In the case of ultra-sensitive analyses such as those reviewed here, the water class required is almost always Type I and frequently Type I+.



*“clearly defined categories of water have been devised so that investigators are able to select the right type of water”*



**Table 1.** The types of water purity required for different applications, in ascending levels of purity

Type III	This is the grade recommended for non-critical work, which may include glassware rinsing, water baths, autoclave and disinfectant feeds, as well as environmental chambers and plant growth rooms. These systems can also be used to feed Type I systems.
Type II	Type II water is employed for general laboratory use. This may include media preparation, the creation of pH solutions and buffers, and for certain clinical analyzers. It is also common for Type II systems to be used as a feed to a Type I system.
Type II+	This is the grade for general laboratory applications requiring higher inorganic purity afforded by standard Type II water.
Type I	Often referred to as ultrapure, this grade is required for highly sensitive applications such as HPLC mobile phase preparation, as well as blanks and sample dilution for other analytical techniques including Gas Chromatography (GC), Atomic Absorption Spectrophotometry (AAS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Type I is also required for molecular biology applications as well as other sensitive techniques such as mammalian cell culture and IVF (in vitro fertilization).
Type I+	Goes beyond the purity requirements of Type I water and is used in processes requiring the highest levels of water purity.

**Table 2.** The physicochemical properties of the different water types available.

	Resistivity (MΩ-cm)	TOC (ppb)	Bacteria (CFU/ml)	Endotoxins (EU/ml)
Type III	>0.05	<200.0	<1000.0	N/A
Type II	>1.0	<50.0	<100.0	N/A
Type II+	>10.0	<50.0	<10.0	N/A
Type I	>18.0	<10.0	<1.0	<0.03
Type I+	18.2	<5.0	<1.0	<0.03

# Working at the limits of detection

Modern research continues to push through the barriers of detection in the pursuit of improving healthcare, informing government policy and protecting the environment. We now frequently make use of equipment to determine concentrations down to the sub-ng/L range – that's less than one part per trillion, the equivalent of finding one person in over 140 Earth-sized populations!

Researchers conducting elemental analysis, for example, have several techniques at their disposal. The current approximate detection limits of some of the commonly employed techniques are as follows:

- ICP-MS: <1 part per trillion
- Graphite Furnace Atomic Absorption Spectrometry (GFAAS): <1 part per billion
- Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES): 1–10 parts per billion
- Flame Atomic Absorption Spectrometry (FAAS): <1 part per million

Trace element detection using techniques such as these is highly dependent upon having a high quality blank or calibration sample, as well as ensuring that the water used in sample preparation is of the appropriate purity. The presence of various molecular species in the solvent (usually water) can cause peak or band structures in the data when using ICP-AES, whereas background contamination during ICP-MS can greatly affect the limit of detection, referred to as the 'background limit', which is especially significant at the level of parts per trillion.

Put simply, if you want to work at the limits of analytical detection, a consistent, reliable source of ultrapure water is an essential part of your workflow.

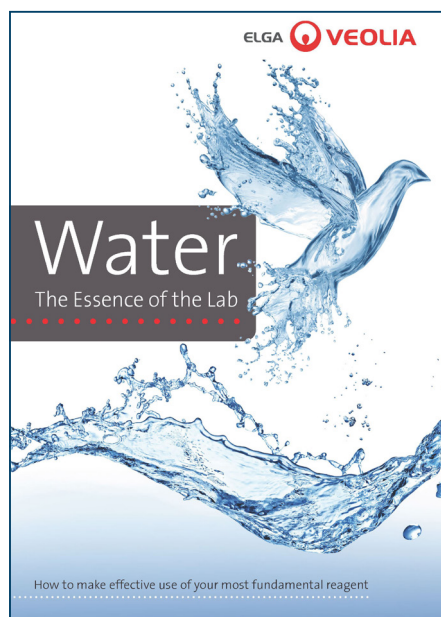




# Matching purity with parameters

Selecting the right analytical technique is dependent upon the type of research being conducted, which in turn will influence the quality of water required. In addition to absolute detection limits, it is worthwhile considering the range of permissible error and level of tolerable contamination your particular research goals will allow. For example, the calibration of an ICP-MS system will require the use of ultrapure water, whereas a routine assay utilizing a spectrophotometer is unlikely to need such high purity. Not only is this knowledge important for ensuring the generation of high quality data, it will also greatly influence the costs of running the lab.

To quickly match your intended application with a recommended water type (and associated acceptable levels of organic content, nucleases, endotoxins, etc.), please refer to our earlier whitepaper – **Water: The essence of the lab.**



[www.elgalabwater.com/wp-water-essence-lab](http://www.elgalabwater.com/wp-water-essence-lab)



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# Get in touch

If you would like to learn more about using pure water for highly sensitive analysis, please contact our team of experts. ELGA LabWater has been working in laboratory water purification exclusively for almost 80 years, making us the world leaders in this area.

As an organization, we're committed to ensuring that those working at the bench receive the highest quality professionalism, and water, possible. If you'd like to start a research program operating at the limits of analytical detection, maybe we can help – get in touch now.

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