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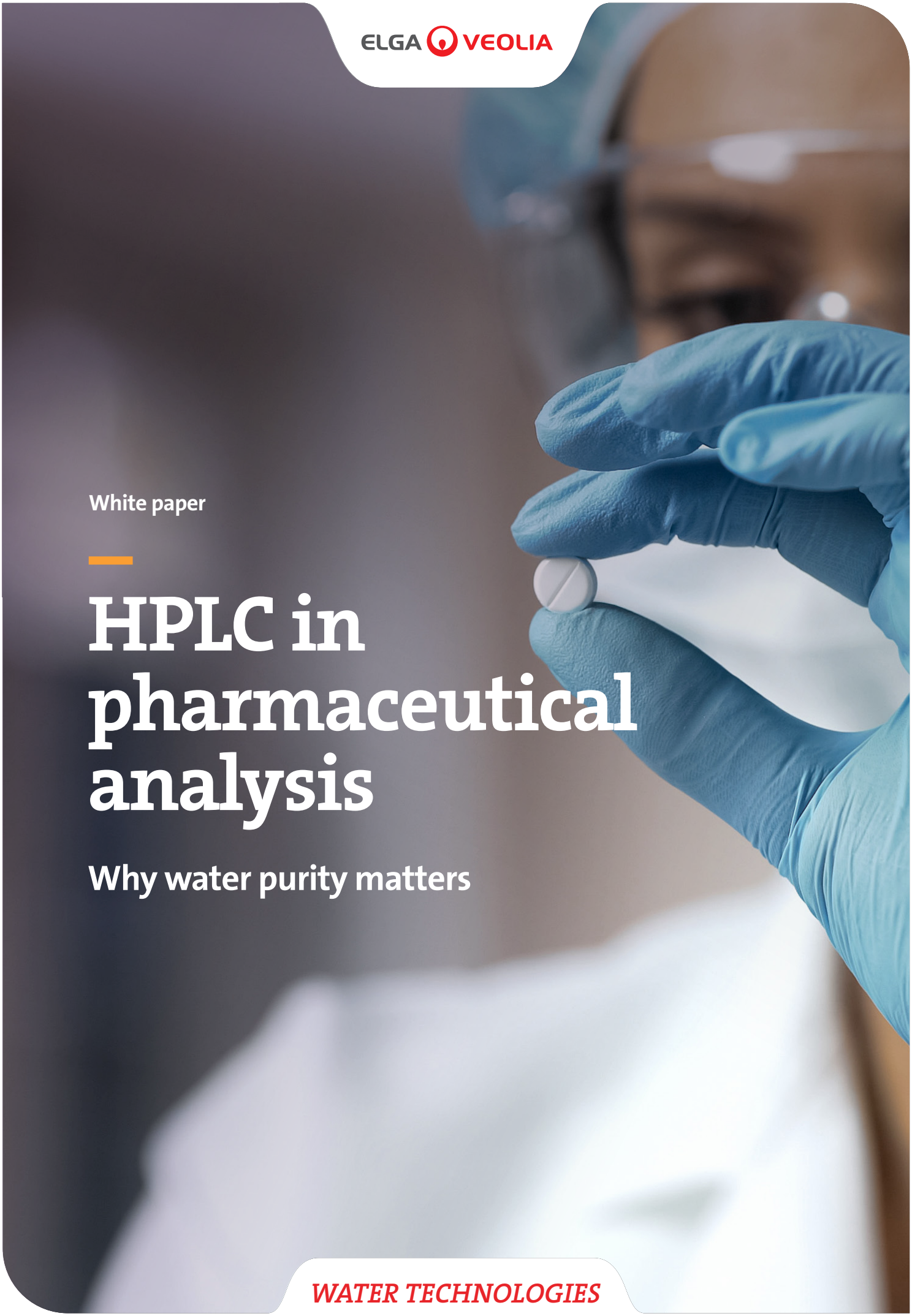


White paper

HPLC in pharmaceutical analysis

Why water purity matters

WATER TECHNOLOGIES

Inside

“Control of the quality of water, in particular the microbiological quality, is a major concern, and the pharmaceutical industry devotes considerable resource to the development and maintenance of water purification systems.”

“Guideline on the quality of water for pharmaceutical use” published by the European Medicines Agency on 13 November 2018

Intro

Water, HPLC and big pharma

Water is essential to the pharmaceutical industry, whether used as a raw material or a solvent in the processing, formulation, manufacture and analysis of pharmaceutical products, notably active pharmaceutical ingredients (APIs) and intermediates.[1]

The quality of the water must be controlled throughout the production, storage and distribution processes, particularly for microbiological and chemical contaminants. The requirements for water quality are dictated by strict, published guidelines, as part of the United States Pharmacopoeia (USP) and the European Pharmacopoeia (EP), amongst others.[2,3]

The consequences of using impure water, contaminated either by bacteria or trace amounts of potentially toxic chemicals may be dire. In the best-case scenario, they may change the concentration of the active ingredient in the final formulation, and in the worst scenario could even result in patient death.[4]

With the widespread production of pharmaceuticals came the legislation to ensure controlled production and purity of drugs.[4] **One of the most common techniques used in pharmaceutical production to verify drug purity globally is high performance liquid chromatography (HPLC).**[5]

HPLC is widely used in the following pharmaceutical areas:

- **Qualitative analysis** - separation of thermally unstable chemical and biological compounds, e.g., drugs (aspirin and ibuprofen), salts (sodium chloride), proteins (egg white or blood), organic chemicals (polystyrene and polyethylene), herbal medicines and plant extracts.
- **Quantitative analysis** - to determine the concentration of an active compound in a sample by measuring the height and/or area of the chromatographic peak.
- **Preparation of pure substances** for clinical and toxicology studies and in organic synthesis (preparative HPLC).
- **Trace analysis** of compounds present in very low concentrations in a sample, useful in pharmaceutical, toxicology, environmental, and biological studies.

HPLC is much more efficient than previously used methods to determine drug purity, e.g. multiple crystallization, which required comparatively large quantities of drug for analysis, thus concurrently minimising losses in pharmaceutical manufacture.[5]

HPLC is not only used for analysis of the finished drug products. Since it can separate compounds, it is also used during manufacture. HPLC can thus provide critical starting products for the manufacture of new drugs as well as aid the characterisation of molecules (lead compounds) that have the potential to be manufactured into drugs.

HPLC can also be used to separate enantiomers, molecules which are mirror images of each other, using chiral stationary phases (CSPs). The ability to prove the purity of enantiomeric molecules, a standard procedure in pharmaceutical assays, is thus one for which HPLC is particularly suited. The most popularly used CSPs in pharmaceutical chemistry are polysaccharide benzoate and phenylcarbamate derivatives.[6]

ELGA EDITORIAL TEAM

intro.

WHICH WATER GRADE SHOULD I USE?

Challenge: maintain the correct level of water purity at different stages of drug manufacture

To adhere to chemical and microbiological quality standards, the feed water for pharmaceutical processes, including for HPLC separation and analysis, must meet the requirements of the National Primary Drinking Water Regulations (NPDWR; 40 CFR 141), or the water regulations of the World Health Organisation drinking water guidelines.[1]

The grade of water quality required during manufacture will depend on the administrative route of a particular drug. For example, the European Pharmacopoeia and United States Pharmacopoeia provide quality standards for grades of water for pharmaceutical use. These are divided into Water for Injections (WFI), Purified Water, and Water for preparation of extracts.[2,3]

Until April 2017, the production of Water for Injections (WFI) had been limited to production by distillation only. In effect, the Ph. Eur. monograph for Water for Injections (0169) was revised in order to allow the production of WFI by a purification process equivalent to distillation, such as reverse osmosis coupled with appropriate techniques such as electro-deionisation, ultrafiltration or nanofiltration.

The revised monograph was published in the Ph. Eur. Supplement 9.1, bringing the Ph. Eur. more closely in line with the US Pharmacopeia and the Japanese Pharmacopoeia, and allowing production of WFI by distillation or by a purification process proven "equivalent or superior to distillation", and "by distillation or by reverse osmosis and/or ultrafiltration", respectively.

This means that water purification systems can now be used to produce water for pharmaceuticals and pharmaceutical analysis, in place of distillation, resulting in lower costs and more sustainable water production, distillation being a very energy-hungry process in comparison.[7]

intro.

WHICH WATER GRADE SHOULD I USE?

Challenge: keep the level of water contaminants down during the pharmaceutical process

We shall now look at the requirements for different types of water at different points in the pharmaceutical process, including that used for HPLC analysis.

QUALITY OF WATER FOR PHARMA USE

Product licensing authorities specify the minimum grade of water that must be used during the manufacture of the different dosage forms or for different stages in washing, preparation, synthesis, manufacturing or formulation.

The grade of water should take into account the nature and intended use of the intermediate or finished product, and the stage in the manufacturing process at which the water is used. Validation and qualification of water purification, storage and distribution systems are all a fundamental part of GMP and form an integral part of the GMP inspection. The criteria for the different water grades are outlined in Table 1.[3]

For guidance, we provide you here with some general examples of the application of these different "grades" of water in pharmaceutical analysis, depending where in the process that the water is used:

1. Water present as an excipient in the final formulation

2. Water used during manufacture of active pharmaceutical ingredients and medicinal products

3. Water used for cleaning/rinsing of equipment, containers and closures.

Parameter	Purified Water		Highly Purified Water		Water for Injection	
	USP	Ph Eur (bulk)	USP	Ph Eur (bulk)	USP	Ph Eur (bulk)
TOC (ppb C)	500	500	NA	500	500	500
Conductivity @ 20°C	NA	≤ 4.3 µS/cm	NA	≤ 1.1 µS/cm	NA	≤ 1.1 µS/cm
Conductivity @ 25°C	≤ 1.3 µS/cm	NA	NA	NA	≤ 1.3 µS/cm	NA
Nitrate (NO3-)	NA	≤ 0.2 ppm	NA	≤ 0.2 ppm	NA	≤ 0.2 ppm
Heavy Metals (ppm as Pb)	NA	≤ 0.1 ppm	NA	NA	NA	NA
Aerobic Bacteria	≤ 100 CFU/ml	≤ 100 CFU/ml	NA	≤ 10 CFU/100ml	≤ 10 CFU/100ml	≤ 10 CFU/100ml
Bacterial Endotoxins (EU/ml or IU/ml)	NA	NA	NA	≤ 0.25	≤ 0.25	≤ 0.25

Different standards of water

Table 1

i.

WATER PRESENT AS AN EXCIPIENT IN THE FINAL FORMULATION

Challenge: ensure there is sufficient water of the correct purity level, depending on intended use of final medicinal product

Water is the most commonly used excipient in medicinal products. The minimum quality of water selected depends on the intended use of the product.

Table 2 summarises the main categories of sterile products. **WFI is required for those products intended for parenteral administration.** For convenience the pharmaceutical industry often uses WFI for the preparation of ophthalmic, sterile nasal/ear and cutaneous preparation. In such situations, highly purified water represents a useful alternative, with the added advantage of satisfying the industry's need for large volumes.

With the exception of some nebuliser preparations, **purified water is the acceptable grade of water for non-sterile products**, as outlined in Table 3.

Sterile medicinal products	Minimum acceptable quality of water
parenteral	WFI
ophthalmic	Highly Purified water
Hemofiltration solutions	WFI
Haemodiafiltration solution	WFI
Peritoneal dialysis solution	WFI
Irrigation solution	WFI
Nasal/ear preparations	Highly Purified water
Cutaneous preparations	Highly Purified water

Categories of sterile products *Table 2*

Non-sterile medicinal products	Minimum acceptable quality of water
Oral preparations	Purified
Nebuliser solutions	Purified*
Cutaneous preparations	Purified**
Nasal/ear preparations	Purified
Rectal/Vaginal preparation	Purified

Water type for non sterile products *Table 3*

*In certain disease states eg. Cystic fibrosis, medicinal products administered by nebulisation are required to be sterile and nonpyrogenic. In such cases WFI or sterilised highly purified water should be used.

**For some products such as veterinary teat dips it may be acceptable to use potable water where justified and authorised taking account of the variability in chemical composition and microbiological quality.

ii.

WATER USED DURING MANUFACTURE OF ACTIVE PHARMACEUTICAL INGREDIENTS AND MEDICINAL PRODUCTS*

*excluding water present as an excipient in the final formulation.

Challenge: use pure water at all stages in the synthesis and formulation of APIs

The acceptable grade of water will depend heavily on the stage at which it is to be used during manufacture, the subsequent processing step, and the nature of the final product.

Tables 4 and 5 summarise the acceptable quality of water for manufacture of active pharmaceutical ingredients (APIs), and for sterile and non-sterile medicinal products.

Manufacture	Minimum acceptable quality of water
Granulation	Purified*
Tablet coating	Purified
Used in formulation prior to non-sterile lyophilisation	Purified
Used in formulation prior to sterile lyophilisation	WFI

Quality of Water used during manufacture of medicinal products *Table 4*

Type of manufacture	Product requirements	Minimum acceptable quality of water
Synthesis of all intermediates of APIs prior to final isolation and purification steps	No requirement for sterility or apyrogenicity in API or the pharmaceutical product in which it will be used.	Potable water*
Fermentation media	No requirement for sterility or apyrogenicity in API or the pharmaceutical product in which it will be used.	Potable water*
Extraction of herbals	No requirement for sterility or apyrogenicity in API or the pharmaceutical product in which it will be used.	Potable water**
Final isolation and purification	No requirement for sterility or apyrogenicity in API or the pharmaceutical product in which it will be used.	Potable water*
Final isolation and purification	API is not sterile, but is intended for use in a sterile, non-parenteral product	Purified water
Final isolation and purification	API is sterile and not intended for parenteral use	Purified water
Final isolation and purification	API is not sterile, but is intended for use in a sterile, parenteral product	Purified water with an endotoxin limit of 0.25EU/ml and control of specified organisms.
Final isolation and purification	API is sterile and apyrogenic	Water for injection

Quality of Water used during the manufacture of active pharmaceutical ingredients (APIs) *Table 5*

*Purified water should be used where there are technical requirements for greater chemical purity; **the application would need to demonstrate that potential variations in the water quality, particularly with respect to mineral composition, would not influence the composition of the extract.

iii.

WATER USED FOR CLEANING/RINSING OF EQUIPMENT, CONTAINERS AND CLOSURES

Challenge: use the right water type for all pharma equipment and packaging

In general, the final rinse of the equipment, containers/closures should be with the same quality as used in the final stage of manufacture of the API or used as an excipient in a medicinal product.

Cleaning/rinsing of equipment, containers, closures	Product type	Minimum acceptable quality of water
Initial rinse	Intermediates and API	Potable water
Final rinse	API	Use same quality of water as used in the API manufacture
Initial rinse including clean in place (CIP) of equipment, containers and closures, if applicable	Pharmaceutical products-non sterile	Potable water
Final rinse including CIP of equipment, containers and closures, if applicable	Pharmaceutical products-non sterile	Purified water or use same quality of water as used in manufacture of medicinal product, if higher quality than purified water
Initial rinse* including CIP of equipment, containers and closures, if applicable	Sterile products	Purified water
Final rinse** including CIP of equipment, containers and closures, if applicable	Sterile non-parenteral products	Purified water or use same quality of water as used In manufacture of medicinal product, if higher quality than purified water
Final rinse** including CIP of equipment, containers and closures, if applicable	Sterile parenteral products	WFI***

*some containers, eg. Plastic containers for eye drops may not need an initial rinse, indeed this may be counter-productive since particulate counts could be increased as a result. In some cases e.g. blow-fill-seal processes rinsing cannot be applied; **If equipment is dried after rinsing with 70% alcohol, the alcohol should be diluted in water of the same quality as the water used for the final rinse; ***Where a subsequent depyrogenisation step is employed the use of highly purified water may be acceptable subject to suitable justification and validation data.

Table 6

iv.

USE OF PURE WATER FOR HPLC IN DRUG MANUFACTURE AND QA

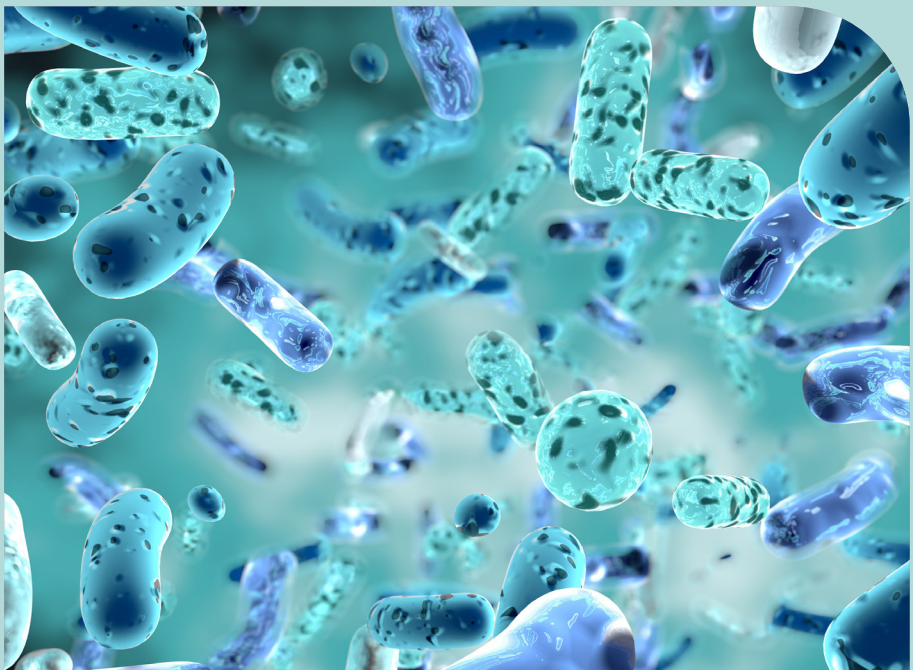
Challenge: avoid false and irreproducible results in your HPLC

When it comes to the effective use of HPLC in the drug manufacturing process, the purity of the water is key.

Water that is not purified to the highest standards can lead to ghost peaks, blocked chromatography columns, poor separations, and more: indeed, most difficulties with HPLC are a result of column problems, in turn due to poor quality lab water.[8]

Virtually all HPLC analyses that are looking for trace impurities require the use of significant quantities of pure water. It is needed throughout the process from sample preparation and pre-treatment, rinse water for solid-phase extraction, reagent water for preparing standards and blanks, to eluent preparation.

In these and other applications the purity of the water can be a critical factor in the sensitivity, reproducibility and robustness of the analytical results obtained. These applications need high quality water with very stringent levels of purity, since even trace levels of impurities can affect one of the stages in HPLC and degrade the quality of a pharmaceutical analysis (Table 7).[9,10,11]



The contamination problems outlined mean that ultrapure water is essential for HPLC analysis, and is key in producing consistent, accurate results. Deionized or distilled water will be insufficient for HPLC in pharmaceutical applications, since it will still contain organic substances and other impurities.

For general pharma lab work, Clinical Laboratory Reagent Water (CLRW) is recommended, which meets the guidelines set forth by the Clinical Laboratory Standards Institute (CLSI) as a minimum requirement.[12]

However, CLRW water is often not pure enough for HPLC, for which Type I (ultrapure) water will be required.

This water reaches extremely high levels of purity, with a resistivity of 18.2 MΩ.cm, total organic carbon (TOC) of <10 ppb, and bacterial count of <1 CFU/ml.[13]

Type of Contamination	Detrimental effect on HPLC
Dissolved organic compounds	Ghost peaks, reduction in sensitivity, reduction in the amount of analyte retained by the column, loss of resolution.
Microorganisms	Blockages in the filters and column, growth of microbes and algae and, release of organic impurities.
Colloids and suspended particles	Can damage the pump. Blockages in the chromatography column can affect performance (e.g. split peaks) and reduce column lifetime.
Ionic species	Can affect the sequence of separation of polar molecules, ghost peaks due to UV-absorbing ions, ionisation effects in detector.
Dissolved nitrogen and oxygen	Bubble formation in the column can disrupt the flow. Gas in the detector can affect the response.

Table 7 The types of contaminants found in water and how these can affect HPLC[8]

Table 7

V.

DISSOLUTION TESTING: A KEY ROLE FOR HPLC IN PHARMACEUTICAL ANALYSIS

Challenge: ensure reproducible bioavailability of APIs from batch to batch

In the pharmaceutical industry, drug dissolution testing is routinely used to provide critical in vitro drug release information for both quality control purposes, i.e., to assess batch-to-batch consistency of solid oral dosage forms such as tablets, and in drug development, in order to predict in vivo drug release profiles.

There are three typical situations where dissolution testing plays a vital role: (i) **formulation and optimisation decisions during product development**, for products where dissolution performance is a critical quality attribute, especially since many drugs are hydrophobic, restricting their bioavailability (ii) **equivalence decisions during generic product development**, and also when implementing post-approval process or formulation changes, similarity of in vitro dissolution profiles between the reference product and its generic or modified version being a key requirements for regulatory approval decisions (iii) **product compliance and release decisions**: during routine manufacturing, where dissolution outcomes are very often one of the criteria used to make product release decisions.[14]

The general procedure for a dissolution test involves a liquid (the dissolution medium) which is placed in standardised vessels within a dissolution unit. Degassing the dissolution medium through sonication or other means is important, since the presence of dissolved gases may affect results. The drug is then added to the medium, and the standardised dissolution protocol is carried out.

Sample solutions collected from dissolution testing are then analysed by HPLC, and the results matched against criteria known as 'release specifications', which samples tested must meet statistically in order for the batch to be accepted for distribution.

In this type of analysis the HPLC requirement is not high sensitivity but good reproducibility.

ENSURING THE QUALITY OF WATER USED IN HPLC ANALYSIS

Commercially available bottled HPLC grade water can be used as an HPLC eluent and for preparing standards and blanks. However, in general, relying on bottled water is inferior to using an in-house purification system, as illustrated in Figure 1.

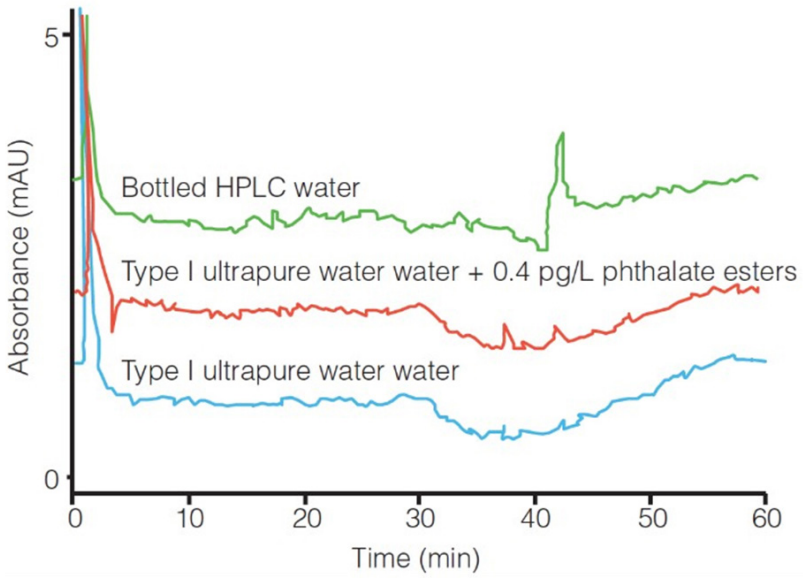
Bottled water can be expensive when significant volumes are used, and the user is dependent on the analyses provided with the bottle, which are usually carried out on bulk sampling. Suitability for specific, individual analyses needs to be confirmed, with controls run on each bottle, and there is a high risk of microbial and particulate contamination once the bottle is opened.

Another study showed that HPLC-grade bottled water resulted in several large peaks, when pre-concentrated on a chromatography column, followed by elution.[10] These peaks were absent when freshly produced ultrapure water was used. When used to separate a drug mixture, bottled water caused large shifts in baselines, as well as ghost peaks.

Again, these problems were not encountered with freshly produced ultrapure water. The authors attributed this to the much higher levels of organic contaminants in bottled HPLC-grade water, when compared to fresh ultrapure water. The use of an in-house water purification system is therefore the best way to minimise the risk of poor quality pharmaceutical analyses.

To find out more about the water purification systems that fulfil the stringent Pharmacopoeia requirements for water that can be used throughout the different stages of the pharmaceutical process, visit www.elgalabwater.com/products

Figure 1



50 ml water concentrated on a C18 column and eluted with a water:acetonitrile gradient, 0–100 % at 5 %/min, flow rate 2 ml/min, with UV detection at 254 nm. Adapted from Reference X.

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