IJPSR (2017), Volume 8, Issue 8 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 10 January, 2017; received in revised form, 10 March, 2017; accepted, 16 April, 2017; published 01 August, 2017

RECENT ADVANCES IN IMPURITY PROFILING OF PHARMACEUTICALS

Anand D. Savkare, Pankaj S. Kalaskar*, Shrikant K. Sarode and Mangesh E. Potkule

MVP Samaj's College of Pharmacy, Nashik - 422002, Maharashtra, India.

Keywords:

Impurities, ICH Q3, Analytical techniques, Drug substance, Drug product

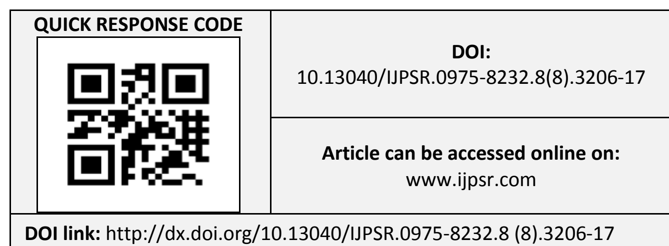
Correspondence to Author:
Pankaj S. Kalaskar

M. Pharm (Quality Assurance Techniques), MVP Samaj's College of Pharmacy, Nashik - 422002, Maharashtra, India.

E-mail: pankajskalaskar@gmail.com

ABSTRACT: Impurities will be present in all drug substances and drug products, *i.e.* nothing is 100% pure if one looks in enough depth. The current regulatory guidance on impurities accepts this, and for drug products with a dose of less than 2 g/day identification of impurities is set at 0.1% levels and above (ICH Q3B (R2), 2006). For some impurities, this is a simple undertaking as generally available analytical techniques can address the prevailing analytical challenges; whereas, for others this may be much more challenging requiring more sophisticated analytical approaches. The present review provides an insight into current development of analytical techniques to investigate and quantify impurities in drug substances and drug products providing discussion of progress particular within the field of chromatography to ensure separation of and quantification of those related impurities. Further, a section is devoted to the identification of classical impurities, but in addition, inorganic (metal residues) and solid state impurities are also discussed. Risk control strategies for pharmaceutical impurities aligned with several of the ICH guidelines, are also discussed.

INTRODUCTION: Pharmaceuticals impurities are the unwanted chemicals that remain with active pharmaceutical ingredients (API) or drug product formulations. The impurities observed in drug substances may arise during synthesis or may be derived from sources such as starting materials, intermediates, reagents, solvents, catalysts, and reaction by - products. During drug product development, impurities may be formed as a result of the inherent instability of drug substances, may be due to incompatibility with added excipients, or may appear as the result of interactions with packaging materials.



QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(8).3206-17

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (8).3206-17

The amount of various impurities found in drug substances will determine the ultimate safety of the final pharmaceutical product. Therefore, the identification, quantitation, qualification, and control of impurities are now a critical part of the drug development process. Various regulatory authorities focus on the control of impurities: the International Conference on Harmonization (ICH), the United States Food and Drug Administration (USFDA), the European Medicines Agency (EMA), the Canadian Drug and Health Agency, the Japanese Pharmaceutical and Medical Devices Agency (PMDA), and the Australian Department of Health and Ageing Therapeutic Goods. In addition, a number of official compendia, such as the British Pharmacopoeia (BP), the United States Pharmacopeia (USP), the Japanese Pharmacopoeia (JP), and the European Pharmacopoeia (EP) are incorporating limits that restrict the impurity levels present in APIs as well as in drug formulations.

The Three Major Categories of Pharmaceutical Impurities: According to ICH guidelines, impurities related to drug substances can be classified into three main categories: organic impurities, inorganic impurities, and residual solvents.

Organic impurities: Organic impurities can arise in APIs or drug product formulations during the manufacturing process or during the storage of drug substances. They may be known, unknown, volatile, or non-volatile compounds with sources including starting materials, intermediates, unintended by-products, and degradation products. They may also arise from racemization, or contamination of one enantiomeric form with another. In all cases they can result in undesired biological activity. Recently, genotoxic pharmaceutical impurities, which may potentially increase cancer risks in patients, have received considerable attention from regulatory bodies and pharmaceutical manufacturers.

In general, genotoxic impurities include DNA reactive substances that have the potential for direct DNA damage. Potential genotoxic impurities include process impurities or degradants, present at trace levels, which are generated during drug manufacturing and storage. As per FDA and EMA guidelines, potential genotoxic impurities are to be controlled at levels much lower than typical impurities. The ICH M7 guidance on genotoxic impurities is currently under preparation with the working title "M7 Assessment and Control of DNA Reactive(Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk".

Inorganic (elemental) impurities: Inorganic impurities can arise from raw materials, synthetic additives, excipients, and production processes used when manufacturing drug products. Several potentially toxic elements may be naturally present in the ingredients and these elements must be measured in all drug products. A further group of ingredients may be added during production and must be monitored for elemental impurities once they are known to havebeen added. Sources of inorganic impurities include manufacturing process reagents such as ligands, catalysts (*e.g.*, platinum group elements (PGE)), metals derived from other stages of production (*e.g*., process water and stainless steel reactor vessels), charcoal, and elements derived from other materials used in filtration. The United States Pharmacopeia (USP) is in the process of developing a new test for inorganic impurities in pharmaceutical products and their ingredients.

The current Heavy Metals Limit Test (USP<231>) is widely acknowledged to be inadequate in terms of scope, accuracy, sensitivity, and specificity, and is due to be replaced with two new general chapters, Limits (USP<232>) and Procedures for Elemental Impurities (USP<233>), due to be implemented in 2013. In parallel with the development of USP<232> and USP<233>, the USP is also introducing a related method <2232> which is specific to dietary supplements. USP<232> defines new, lower permitted daily exposure (PDE) limits for a wider range of inorganic elemental impurities: As, Cd, Hg, Pb, V, Cr, Ni, Mo, Mn, Cu, Pt, Pd, Ru, Rh, Os, and Ir. A complete list of regulated elements and PDEs can be found in Agilent publication 5990-9365EN and the references therein. USP<233> further defines the sample preparation and method validation procedures that should be used for system suitability qualification of any instrumentation used for the analysis of elemental impurities in pharmaceutical materials. Validation of analytical instruments that are used for the new USP<232> and USP<233> methods will be performance based. USP<233> defines the analytical and validation procedures that laboratories must use to ensure that the analysis is specific, accurate, and precise.

Residual solvents: Residual solvents are the volatile organic chemicals used during the manufacturing process or generated during drug production. A number of organic solvents used in synthesis of pharmaceutical products have toxic or environmentally hazardous properties, and their complete removal can be very difficult. In addition, the final purification step in most pharmaceutical drug substance processes involves a crystallization step which can lead to the entrapment of a finite amount of solvent which can act as a residual impurity or can cause potential degradation of the drug. Residual solvent levels are controlled by the ICH, USP, and EP.

Depending on their potential risk to human health, residual solvents are categorized into three classes with their limits in pharmaceutical products set by ICH guidelines Q3C. The use of class I solvents, including benzene, carbon tetrachloride, 1, 1-dichloroethane, 1, 2-dichloroethylene, and 1, 1, 1–trichloroethane, should be avoided. Class II solvents, such as methanol, pyridine, toluene, N, N-dimethylformamide, and acetonitrile have permitted daily exposure limits (PDEs). A few examples of common organic solvents which are found as volatile impurities and have their limits set by ICH guidelines are depicted in **Table 1**. Class III solvents, such as acetic acid, acetone, isopropyl alcohol, butanol, ethanol, and ethylacetate should be limited by GMP or other quality-based requirements.

TABLE 1: ICH LIMITS FOR A SELECTED LIST OF COMMON ORGANIC SOLVENTS FOUND AS VOLATILE IMPURITIES

Volatile Organic Impurity	Limit (ppm)	PDE (mg/day)
Acetonitrile	410	4.1
Chloroform	60	0.6
1,4-Dioxane	380	3.8
Methylene Chloride	600	6.0
Pyridine	200	2.0
1,1,2-Trichloroethane	80	0.8

USP <467> 2009 General Chapter contains a more comprehensive method for residual solvent analysis that is similar to the ICH guidelines developed in 1997. Here, a limit test is prescribed for class 1 and class 2 solvents while class 2C solvents are usually determined by non-headspace methods due to their higher boiling point. The limits of detection (LOD) recommended for class 3 solvents are up to 5000 ppm. When the levels of residual solvents exceed USP or ICH limits, quantitation is required [1].

Regulatory Framework for Controlling Impurities: Impurities are controlled within the framework of the International Conference of Harmonisation (ICH) quality guidelines (ICH Q3A, Q3B, Q3C, Q3D, Q6A, and Q6B) and the multi-disciplinary guidance (ICH M3 and M7). The latter will not be discussed here as it is the focus of a separate review in this issue. ICH Q3A provides guidance on the content, identification and qualification of impurities in new APIs produced by chemical syntheses. Organic impurities can arise during API manufacturing process or subsequent storage. They can be classified as either identified or unidentified, and include starting materials, reagents, by-products, intermediates, filter aids, and degradation products. The guidance describes classification and identification of API impurities, the listing of impurities in specifications (see also ICH Q6A, ICH Q6B), and relevant analytical procedures. In addition, the guidance includes suitable advice for qualifying impurities using appropriate safety studies, in batches of a new API used in safety and clinical studies. Controlling the stereochemical or enantiomeric purity of an API should also be performed in a similar manner to the control of other achiral impurities [2].

Similarly, ICH Q3B provides guidance on the content, identification and qualification of impurities in new drug products. ICH Q3B has a comparable scope to ICH Q3A. Impurities in new drug products (or degradation products) arise as a result of manufacture or storage. They are typically degradants of the API or reaction products of the API with a processing aid or with an excipient (or an impurity within an excipient) or with the immediate primary packing materials. ICH Q3C recommends the acceptable amounts of residual solvents that are allowable in APIs and drug products.

The optimal selection of the solvent for the synthesis of an API may improve the yield, or define important physicochemical characteristics of API such as crystal form, purity, and solubility. Therefore, the solvent may sometimes be a critical process parameter (CPP) within the synthetic process. The guideline describes those levels considered to be toxicologically acceptable for some residual solvents, which are based on permitted daily exposure (PDE) limits and recommends the use of less toxic alternatives, *i.e.* avoidance of class 1 toxic solvents such as benzene. ALARP considerations based on process capability are required for class 3 solvents, but are specifically excluded for class 2 solvents. However, the reality is that regulators will expect process capability considerations to be applied to all classes of solvent. ICH Q3D recommends the acceptable amounts of residual elemental impurities that are allowable in drug products.

Elemental impurities may arise from several sources. The principal source is residual catalysts arising from the use of catalysed coupling reactions (often late stage) during API synthesis. These approaches are environmentally friendly in nature and typically decrease the cost of goods (CoGs) by making the process more efficient and increasing yields. Alternatively, elemental impurities may arise as API or drug product impurities; for example, drug product impurities in excipients, leachable from primary or secondary processing equipment or from packing materials. The guidance covers the evaluation of the toxicity data for potential elemental impurities; the establishment of PDEs (permitted daily exposures) for each elemental impurity, and application of a risk-based approach to control those impurities in drug products. ALARP considerations based on process capability evaluations are not specifically required and the safety based limits alone are deemed to be acceptable.

Analytical Advances: The prime considerations for any analytical method are that it exhibits appropriate specificity and sensitivity, *i.e.* the method is 'fit for purpose'. The workhorse of impurity profiling still remains reverse phase high performance liquid chromatography (RP-HPLC or more commonly, HPLC); either using the standard ultraviolet (UV) detection mode, favoured within quality control (QC) environments, or the more sophisticated hyphenated mass spectrometry (MS) detection that is primarily used for identification, but sometimes also for routine control purposes. However, within the framework of impurity profiling there are still ample opportunities for improvements based on enhancing specificity and sensitivity, enhancing robustness or improving cost effectiveness.

This has engendered advances in several areas:

- Column technology, *i.e.* monolithic, core shell or low particle size fully porous stationary phases
- Hardware, *i.e.* ultra- high performance liquid chromatography (UPLC or UHPLC)
- Different chromatographic approaches, *i.e.* hydrophilic interaction chromatography (HILIC) for more polar impurities,
- Orthogonal approaches, *e.g*. mixed mode, HILIC and RP-HPLC
- Universal detectors for detection of poorly UV absorbing impurities (for better mass balance),
- Better interfaces between the separation tools and the detection modes
- Enhancement of detection systems, *e.g.* optimisation of source geometry of electrospray ionisation mass spectrometry (ESI-MS) detectors
- Niche applications, *e.g.* capillary electrophoresis (CE), inductively coupled plasma- mass spectrometry (ICP-MS), gas chromatography (GC), etc.

This review will focus on all of these different areas as well as a few additional elements, such as solid stage analysis of impure polymorphic forms.

Column Technologies: While the 'work horse' of columns within the pharmaceutical industry have been typically brands such as XBridge®, Luna® and Zorbax® there is a continuous development of new column materials, which may be used in the pharmaceutical field given that this will offer an advantage over legacy materials. Which are the column materials that will be used in the future is hard to predict as it's a function of many inputs, *i.e.* experience, chemical space of new compounds and thereby the need for another separation, or development of a new organic chemical method to support the quantification of new impurities. This section therefore described the separation of impurities and the role of recent developments in stationary materials and technologies that can facilitate better separation of impurities, which may come into use in the pharmaceutical field.

Sub 2 Micron Phases: Reported [3] that there is a large choice of <2 µm stationary phases available for use in either UPLC (ultra performance liquid chromatography), sometimes referred to as UHPLC (ultra high performance liquid chromatography) or HILIC separation modes. Based on robustness testing they recommended that the following stationary phases offer sufficient flexibility for rapid assessments of most separation problems: Agilent Zorbax RRHD (silica), Aquity BEH (hybrid silica), Thermo Syncronis HILIC (Zwitterionic phase) and Aquity BEH amide (amide).

Assessed [4] the potential for 1.3 µm core shell particles consisting of non-porous cores of 0.9 µm with porous shells of 0.2 µm in thickness, which gave enhanced performance (> 450,000 plates/m versus 300,000 plates/m for a fully porous < 2 µm column). However, only relatively short columns could be utilised due to very high back pressures. The authors indicated that, to fully utilise these innovations in column technology, instruments with higher back pressures would be required (1500-2000 bar versus 1200 bar currently for UHPLC) along with reductions in extra column variance (≤1 µL versus 2 µL currently). The new Phenomenex Kinetic 1.3 µm columns (together with the more established 1.7 and 2.6 µm columns) were used to assess the resolution of two impurities of α-estradiol [5].

All three columns gave similar resolution in < 2.5 minutes, but the efficiencies (N) were in the ration of 1.9/1.2/1.0, respectively and the operating pressures were 856/530/316 bar, respectively. Again, the authors indicated that the pressure limit was too restrictive to obtain the optimal linear velocity, indicating that optimal working pressures were in the region 1500-2000 bar.

Investigated [6] the loading performance of several 2.7 µm shell and sub 2 µm totally porous columns, including one pair of columns manufactured from similar materials with similar bonding chemistries using strongly acidic and basic probe compounds. In general, the capacity of shell particles was not greatly reduced, despite containing a smaller porous volume, *i.e.* limited benefit of the low particle size.

Evaluated [7] a 1.7 µm Whelk-O 1 chiral stationary phase in supercritical fluid conditions for the screening of 129 racemic compounds. Using a 5 cm columns, the authors reported resolutions of more than 2 in more the 65% of the cases and even higher percentages for acid and neutral compounds, demonstrating that this clearly has a potential for high-throughput enatioselective screening. [8] Used sub-2 micron columns in super critical fluid chromatography (SFC) and reported that the increase in back pressure due to the reduced particle size did not require significant modifications to operate within the subcritical domain. [9] Used a sub-2 micron column for separation in subcritical fluid chromatography for the analysis of steroids and reported that this variation of SFC also benefit from the use of sub-2 micron columns.

Fused Core Stationary Phases: Fused core stationary phases have particle diameters of 2.7µm with a 0.5µm porous layer overlaid on top of 1.7µm solid core [10]. Although, the sample loading capacity is reduced by about one quarter due to the solid core, the operating pressures are reduced by about 60% allowing them to be used with conventional HPLC pumps (see UHPLC). Mass transfer properties are increased resulting in higher flow rates with minimal impact on efficiencies. Indeed, [11] have demonstrated that these stationary phases give similar efficiencies to sub 2 micron columns. There are a variety of fused core shell stationary phases available, including AMT Halo, Supelco Ascentis Express, Agilent Poroshell and Phenomenex Kinetex.

Demonstrated [12] rapid analysis (< 5 minutes), excellent efficiency (ca. 14,000 plates), and reproducibility (<1%RSD) over 500 injections using torcetrapib as the analyte of choice. Resolution between torcetrapib and impurity D was also maintained (<2.7%RSD). Developed [13] a UHPLC method for in process monitoring of reaction mixtures, where rapid resolutions in short time frames were a pre-requisite and standard HPLC equipment could be used. The authors used an Ascentis Express C18 column with a flow rate of 2.5ml/minute. The run time was 6 minutes.

Compared [14] totally porous (UPLC) and core shell (UHPLC) approaches for optimising the resolution of enantiomers, with a polysaccharide chiral selector. In all cases better resolution was obtained using the core shell approach. For separation of synthetic peptides and tryptic digests. [15]Also reported fused core particle materials comparable to sub 2 µm particle, but with less than one half of the operating back pressure.

Evaluated [16] the performance of reversed-phase and ion pairing chromatography coupled with UV detection for the analysis of a set of 12 catecholamines and related compounds. Different chromatographic columns (porous C18-silica, perfluorinated C18-silica, porous graphitic carbon,

monolithic and fused-core silica-based C18 columns) were tested using semi-long perfluorinated carboxylic acids as volatile ion-pairing reagents. Much more promising results were reported with ion pairing chromatography than reverse-phase chromatography and further important improvements in analytes peak symmetry and separation resolution were observed when using the monolithic and fused-core C18 columns under IPLC conditions. For UV detection, a satisfactory separation of the 12 selected analytes was achieved in less than 20 min by using a fused-core particle column.

Monolithic Stationary Phases: These type of columns use a single, porous monolithic stationary phase, *i.e.* rods. They are comprised of small mesopores allow appropriate retention and selectivity, together with large flow through macro pores (2µm). This complementary pore structure delivers good permeability, lower flow resistance resulting in higher flow rates with moderate back pressures, in comparison with standard particle based columns of similar efficiencies [17]. They have been mostly utilised in high throughput applications. They can be sub-divided into (i) inorganic, (ii) and organic polymers. Both types of column can be used in the HILIC operating mode. Some of the newer polymeric monoliths can demonstrate different retention mechanisms, *i.e.* with the appropriate mobile phase they can be operated in reverse phase, HILIC, and potentially ion-exchange (IEX) or size exclusion (SEC) modes[18].

Monolithic Stationary Phases (Silica Based): These columns have similar surface area to conventional particle packed columns [19], a bi-modal pore structure with a high percentage of mesopores (712nm). Silica monoliths are ideal for small molecule applications with high efficiencies, higher flow rates and lower operating pressures [20]. In addition, they can also be chemically modified. The newer, 2nd generation columns, *i.e.* Merck Chromolith or Phenomenex Onyx, have better radial pore distribution, with performances similar to sub 3-micron columns.

Developed [21] a fast assay and impurity method for the in-process monitoring of aprepitant API using a Merck Chromolith performance RP-18e column with a flow rate of 5.0ml/minute. The method achieved resolution of the API and four related impurities in <2 minutes. The run time was reduced by 14x compared to the established HPLC method. Monolithic columns have also seen widespread usage in dissolution testing using UHPLC, where samples numbers are large and need to be analysed rapidly. Tzananvaras and co-workers developed fast HPLC stability indicating methods (< 60 seconds) for nimesulide [22] and selegine [23].

Monolithic Stationary Phases (Polymer Based): The most common polymeric monoliths are based on polyacrylamide, polymethacrylate and polystyrene copolymers. These stationary phases show heterogeneous micro structure, with relatively low surface areas. The mesopores swell in the presence of mobile phase and can retard small organic solutes; equally, biomolecules can be retained by convection mechanisms in the 'through-pores', *i.e.* they have size exclusion possibilities [24]. They demonstrate broad chemical variability, as well as high pH and temperature stability.

Diamond based stationary phases: Porous graphitic carbon (PGC) has been used for many years in separation sciences, but more recently diamond has been proposed as an alternative phase, particularly for HTLC (high temperature liquid chromatography) and UPLC at very high pressures. This is because diamond combines thermal, hydrolytic and mechanical stability, excellent thermal conductivity, aligned with no thermal expansion and limited swelling or shrinking in the presence of solvents, together with chemical inertness. Additionally, the surface can be readily modified to yield hydrophilic or hydrophobic properties [25]. Owing to the non-porous particle nature, columns packed with high pressure diamond stationary phases may exhibit excellent mass transfer and produce separations with maximum column efficiency of 128,200 theoretical plates per meter [26].

High Temperature Compatible Phases: Stationary phases that are suitable for HTLC include silica columns containing either modified, polydentate (Blaze C8 or C18) or hybrid silica (XBridge C18), metal oxide based phases, *i.e.* zirconia (Zirchrom-PS, -PBD, -DB, -Carb), organic

polymeric (Hamilton PRP-1), diamond and porous graphitic based columns (Hypercarb) [27, 28].

Chiral Stationary Phases: Due to the different pharmaceutical properties of enantiomers, chiral resolution has gained significant focus both from an analytical and regulatory perspective [29]. Assessed [30] 25 different stationary phases to resolve a training set of 80 chiral molecules, using SFC (super critical flu0id chromatography). The columns were divided into 8 different classes, based on column chemistries, summarised in **Table 2**.

TABLE 2: SUMMARY OF DIFFERENT CHIRAL STATIONARY PHASES [30]

Column Chemistry	Column Names
Cellulose tris (4-methylbenzoate)	Chiracel OJ, Cellulose-3
Cellulose tris (3,5 dimethylphenylcarbamate)	Chiracel OD, Lux Cellulose-1, Epitomize IC, Chiralpak 1B*
Amylose tris (3,5 dimethylphenylcarbamate)	Chiralpak AD, Chiralpak IA*, Epitomize 1A
Cellulose tris (3-chloro-4 methylphenylcarbamate)	Cellulose-2, Chiracel OZ
Cellulose tris (4-chloro-3 methylphenylcarbamate)	Cellulose-4, CC4
Amylose (5-chloro-2 methylphenylcarbamate)	Amylose-2, Chiralpak AY, Chiralpak IE*, Chiralpak ID*, Chiralpak IF*
Cellulose tris (3,5 dichlorophenylcarbamate)	Chiracel IC*
Cellulose tris (3-chloro-4 methylphenylcarbamate) / Cellulose tris (3,5-dichlorophenylcarbamate)	CCC
1-(3,5-dinitrobenzamido) tetrahydrophenanthrene	Whelk 01
Cyclofructan	Larhic CXF6, Larhic CF6-N
Teicoplanin (antibiotic phase)	Chirobiotic T2

Indicated [30] that all 80 chiral compounds they were investigating could be partially separated and 65/80 could be baseline separated using all 25 chiral stationary phases and the 3 different mobile phases; however, this is an impractical approach. Using a sub-set of 6 chiral stationary phases (AD, AS, AY, CC4, ID, and Whelk 01) gave partial separation of 78/80 compounds and full separation of 51/80 using a single mobile phase (ethanol/0.1% ammonia). Then the authors use a second set of chiral columns (CCC, Cellulose-3, OJ, IA, IE, and IF) and the 3 mobile phases to get 80/80 partial separations and 64/65 full resolutions.

Sub 1μm mesoporous silica derivatised with cyclodextrin chiral separators used at moderate operating pressures (<8000 psi) and reasonably high flow rates (2.0 ml/min.) were used to resolve some representative chiral drugs in less than 10 minutes [31]. Given the increased need for chiral analysis after general introduction of chiral drug molecules to the market much effort have been put into development of chiral columns. The price of the materials have therefore decreased significantly and a huge amount of science have been dedicated to this field, why the interested reader is directed to but a few among many reviews in the field [29, 32 33, 34, 35].

Developments in chromatographic approaches: Developments in equipment and methods to conduct chromatographic separations have meant new analytical opportunities for fast and/or efficient separations and thereby analysis of impurities. Many of these developments have matured such that they are now commercially available, for example, UPLC is considered as a standard methodology in most research and development laboratories. This section will describe this in detail as well as other technological advances in the analysis of impurities in pharmaceutical products.

Ultra-High Performance Liquid Chromatography (UHPLC): Ultra-High Performance Liquid Chromatography (UHPLC) is effectively a 'half-way' house between conventional HPLC and UPLC (although rather confusingly, some research groups also refer to very high pressure UPLC as UHPLC). In this article we will use the terminology UHPLC, where sub-2 micron columns are used to provide enhanced speed, whilst trying to continue to use conventional HPLC equipment [36]. In terms of transferring methodology between HPLC (3.5μm) and UHPLC (1.7 μm) equivalency can be maintained by reducing the column length by 50% and doubling the flow rate. However, it isn't typically possible to use conventional HPLC equipment with sub 2 micron columns as the pressure increases by 4-fold and is above the safe working threshold of most HPLC pumps. The extra-column broadening is increased due to the higher system volumes in standard HPLC systems and this decreases peak resolution compared to that obtained in a UHPLC instrument. Finally, as the

peak widths decrease by one-quarter the data collection systems need to increase by 4-fold to compensate. To address these problems fused core stationary phases are typically utilised [36].

Developed [38] UHPLC methods for an injectable lidocaine product using 1.9µm Hypersil GOLD column with a flow rate of 1.6ml/minute. The method had a run time of 0.4 minutes, which was 10-fold faster than the conventional HPLC method with equivalent resolution. Similarly, [39] developed an isocratic UHPLC method for the analysis of primaquine phosphate drug substance and related impurities using a 1.7µm Water Acquity BEH C18 column, with a flow rate of 0.5ml/minute. The method was considerably faster (10-fold) than the existing HPLC method with similar resolution of the related impurities. The method was satisfactorily validated.

Ultra Performance Liquid Chromatography (UPLC): Due to the varying structures/polarities encountered during impurity profiling, complex gradients of 30 minutes, or more, are not uncommon with classical HPLC [40]. This generated an impetus to significantly reduce separation times, without impacting on the selectivity of the method, and in some cases, enhancing the selectivity; but this in turn requires enhanced separation efficiency. Ultra performance liquid chromatography (UPLC) was first introduced during 2004 by the Waters Corporation. UPLC uses sub 2 µm column packings for enhanced separation efficiencies, but this necessitates significantly increasing the instrument's ability to function at higher operating pressures (up to 15000 psi, *i.e.* 1000 bar), which in turn require bespoke operating equipment (particularly pumps and valves).

Recent synthetic chemistry developments are producing increasingly complex impurity profiles, *i.e.* positional isomers, regioisomers, diastereomers, etc., that can be extremely challenging to resolve and accurately quantify [41]. It is still unclear whether this increasing synthetic complexity will necessitate novel, 'next generation', analytical approaches (developed for natural products, proteomics, etc.), or whether the existing developments in column technologies, equipment, operating modes *etc*., will adequately address the challenge. [41] Tried to answer this key question by attempting to resolve warfarin from its five mono-hydroxylated isomers. The authors used UPLC with six sub-2µm stationary phases (1.8µm Zorbax Eclipse Plus C18, 1.8µm Zorbax Eclipse Plus SB-8, 1.8µm Zorbax Eclipse Plus SB-CN, 1.8µm Zorbax Eclipse Plus SB-phenyl, 1.9µm Hypersil Gold AQ, and 1.9µm Hypersil Gold PFP) at low (pH 2.7), medium (pH 6.5) and high pH (pH 8.0). The Eclipse C18 column at pH 2.7 gave the best initial results showing some resolution of 4/5 of the isomers. By optimising the gradient, all of the isomers could be resolved using this column in 2.6 minutes, which is significantly faster than published methods.

Developed [42] a UPLC method for riluzole and related impurities using a 1.8µm Waters Acquity HSS T-3 column at 0.9ml/minute. The run time was 3 minutes. Similarly, [43] developed a UPLC method for ropinirole and related impurities in API and drug product using a 1.7µm Waters Acquity BEH column at low flow rates.

Hydrophilic Interaction Chromatography (HILIC): To fully utilise the low back pressures and decreased viscosities generated using the HILIC operating mode, it is advantageous to develop HILIC methods using <2µm stationary phases [3]. This can be done using classical HPLC hardware, rather than any requirement to use UPLC equipment-provided that the extra column volumes and dwell volumes are aligned with the column dimensions [43, 44]. In addition, the frictional heating effects that can bedevil the use of classical HPLC hardware with <2µm stationary phases when working with ultra-high pressures (≤ 1300 bar) is significantly reduced with HILIC as the solvent viscosities and subsequent back pressures are much lower than with RP-HPLC (2-3x less).

Mobile phase pH was also an important parameter impacting on specificity and retention, but the use of high levels of organic modifier can make this parameter difficult to measure. However, the choice of organic modifier, column temperature and ionic strength of buffer appear to have less impact on these important variables [3].

HILIC utilises polar stationary phases (cf. normal phase HPLC) and aqueous-polar organic mobile phases, which contain high levels (>60%) of an

organic modifier, typically acetonitrile [45]. This mobile phase has an intrinsically low back pressure due to its low viscosity, and the superior volatility afforded by the high organic modifier content enhances droplet formation and desolvation efficiencies, which in turn lead to significantly enhanced sensitivity gains in electrospray ionisation mass spectrometry (ESI-MS) [46] or other aerosol based universal detectors, e.g. charged aerosol detection (CAD) or evaporative light scattering detection (ESLD) [47].

A recent comparative assessment of the relative sensitivities of RP-HPLC-ESI-MS versus HILIC-ESI-MS based on signal-to-noise (S/N) ratios of 56 basic drugs of varying physicochemical properties showed an approximately 4-fold enhancement of HILIC versus the more standard RP-HPLC [45]. Interestingly, this same research group [48] reported significantly less sensitivity gains for the more modern triple quadrupole ESI-MS instruments compared to older generation instruments. They reported that this was because modern ESI-MS detectors enhanced the evaporation efficiencies for those mobile phases containing high percentages of water, typically used in the RP-HPLC mode.

High Temperature Liquid Chromatography (HTLC): Liquid chromatography at increased temperatures has been explored as a means of reducing analysis times. Temperatures as high as 200°C have been explored [49]. Temperature programming has been utilised to enhance resolution of poorly resolving analytes [50]. Additionally, hot water as a super critical fluid exhibits lower viscosity, decreased polarity and increased solubilisation capacity and has been used as an isocratic mobile phase for RP-HPLC [51]. The removal of organic solvents also facilitates HPLC-NMR (nuclear magnetic resonance spectroscopy) applications [52].

HTLC tends to be divided into two main approaches. Either, utilising conventional HPLC-type stationary phases and very high temperatures, *i.e.* up to 200°C [53] or using UHPLC/UPLC stationary phases (sub 2µm and high operating pressures, *i.e.* > 400 bar) and temperatures up to 90°C [54]. HTLC has been used to analyse a wide variety of pharmaceuticals. [55] Developed an HTLC method using 3.5µm Zorbax StableBond C18 column at 0.9ml/minute and 90 °C to optimise the resolution of an API and at least 10 impurities. [56] Used a Zorbax rapid resolution HT stable bond C18, 1.8µm, gradient phase, with temperature programmable gradients up to 95 °C. This gave separations in <3 minutes with good specificity.

HTLC with ESI-MS detection was used by [57] for the separation of components of over the counter cold medicines. They assessed four different stationary phases at various pHs, over the temperature range 30 to 90 °C and the best performance was shown using a polymer stationary phase (Polymer Laboratories PLRP-S) at pH 10 and at 90 °C the method was fast, selective, efficient, and with reduced peak tailing for the four analytes studied.

However, HTLC is still not a routine technique within pharmaceutical analysis [58]. This is due to the lack of commercially stable stationary phases that have good stability at the top end of the operating range, *i.e.* ≥200 °C, limited experience, the requirements for specialised heating equipment, as well as limited thermal stability of many analytes (this is particularly important for impurity profiling).

Future trends are likely to involve coupling of several columns in series, *i.e.* 10 x 25-cm, this is because of the low back pressures that can be achieved, due to reduced solvent viscosity at elevated temperatures [55]. In addition, there are clear benefits from combining UHPLC/UPLC with increased temperature to optimise complex resolutions [58, 59, 60].

CONCLUSION: Impurities will always be regarded as a ‘necessary evil’, but with the advent of more safety based limits to control related substances (ICH Q3A-D and M7) there is an increasing acceptance that control strategies based on process understanding will allow an appropriate tolerability of risk (patient needs versus commercial necessities). A recent article by a research group from Merck posed an intriguing question: Does the increasing levels of synthetic complexity necessitate novel, ‘next generation’, analytical approaches or do the existing developments in column technologies, equipment, operating modes adequately address the challenge?

The clear answer is that the current state of the art does provide adequate sensitivity and selectivity. However, selectivity, particularly for drug products where matrix interference is still a significant issue and is still seen as a more significant challenge for pharmaceutical impurities.

However, advances in one area often prompt simultaneous advances in other areas. A good example would be that advances in column science catalysed a new generation of hardware capable of coping with significantly increased back pressures (UPLC) and this in turn catalysed the renaissance of SFC. Then advances in core shell columns allowed 'sub 2 micron' performance to be delivered on existing HPLC platforms leading to the development of UHPLC. These synergistic advances should allow the most complex of separation problems to be addressed using the current evolving technology, without the need for ground breaking advances.

ACKNOWLEDGEMENT: We would like to express heart full thanks to our beloved parents for their support and blessings, our respected teachers for their valuable guidance and friends/classmates for their support and good wishes for the completion of this review article.

CONFLICT OF INTEREST: We have no conflict of Interest.

REFERENCES:

1. Primer, Pharmaceutical Impurity Analysis Solutions, Agilent Technology 4-6
2. De Camp, W.H. The FDA perspective on the development of stereoisomers. Chirality. 1989; 1: 2-6.
3. Periat, A., Debrus, B., Rudaz, S., Guillarme, D. a. Screening of the most relevant parameters for method development in ultra-high-performance liquid chromatography. J.Chromatogr. A. 2013; 1282: 72-83.
4. Sanchez, A.C., Friedlander, C., Fekete, S., Anspach, J. Guillarme, D. Pushing the performance limit of reversed-phase ultra-high performance liquid chromatography with 1.3 µm core-shell particles. J.Chromatogr. A. 2013; 1311: 90-97.
5. Fekete, S., Guillarme, D. Kinetic evaluation of new generation of column packed with 1.3 µm core-shell particles. J. Chromatogr. A. 2013; 1308: 104-113.
6. Fallas, M. M., Buckenmaier, S. M., McCalley, D. V. A comparison of overload behaviour for some sub 2µm totally porous and sub 3µm shell particle columns with ionised solutes. J. Chromatogr. A, 2012; 1235: 49-59.
7. Sciascera, L., Ismail, O., Ciogli, A., Kotoni, D., Cavazzini, A., Botta, L., Szczerba, T., Kocergin, J., Villani, C., Gasparrini, F. Expanding the potential of chiral chromatography for high-throughput screening of large compound libraries by means of sub–2µm Whelk-O 1 stationary phase in supercritical fluid conditions. J. Chromatogr. A, 2015; 1383: 160-168.
8. Khater, S., West, C., Lesellier, E. Characterization of five chemistries and three particle sizes of stationary phases used in supercritical fluid chromatography. J. Chromatogr. A, 2013; 1319: 148-159.
9. Nováková, L., Chocholouš, P., Solich, P. Ultra-fast separation of estrogen steroids using subcritical fluid chromatography on sub-2-micron particles. Talanta, 2014; 121: 178-186.
10. Nováková, L., Lopez, S.A. Solichova, D., Satinsky, D., Kulichova, B., Horna, A., Solich, P. Comparison of UV and charged aerosol detection approaches in pharmaceutical analysis of statins, Talenta, 2009; 78: 834-839.
11. Chester, T.L. Sub 2- micron performance with a conventional HPLC instrument using 2.7-micron fused core particles. American Laboratory. March, 2009; 1-8.
12. De Stefano, J.J., Langlois, T.J., Kirkland, J.J. Characteristics of superficially porous silica particles for fast HPLC: Some performance characteristics with sub 2µm particles. J. Chromatogr. Sci. 2008; 46: 254-260.
13. Salisbury, J.J. Fused-core particles: A practical alternative to sub- 2 micron particles. J. Chromatography. Sci. 2008; 46: 883-886.
14. Abrahim, A., Al-Sayah, M., Skrdla, P., Bereznitski, Y., Chen, Y., Wu, N. Practical comparison of 2.7µm fused-core silica particles and porous sub-2µm particles for fast separation in pharmaceutical process development. J. Pharm. Biomed. Anal. 2010; 51: 131-137.
15. Anali, S., D'Orazio, G., Farkas, T., Chankvetadze, B. Comparative performance of capillary columns made with total porous and core shell particles coated with a polysaccharide-based chiral selector in nano-liquid chromatography and capillary electro chromatography. J. Chromatogr. A. 2012; 1269: 136-142.
16. Schuster, S. A., Boyes, B. E., Wagner, B. M., Kirkland, J. J. Fast high performance liquid chromatography separations for proteomic applications using Fused-Core® silica particles. J. Chromatogr. A, 2012; 1228: 232-241.
17. Chirita, R. I., Finaru, A. L., Elfakir, C. Evaluation of fused-core and monolithic versus porous silica-based C18 columns and porous graphitic carbon for ion pairing liquid chromatography analysis of catecholamines and related compounds. J. Chromatogr. B, 2011; 879: 633-640.
18. Aggarwal, P., Tolley, H.D., Lee, M.L: Monolithic bed structure for capillary liquid chromatography. J. Chromatogr. A. 2012; 1219: 1-14.
19. Jandera, P., Hájek, T., Sandra, P. Monolithic and core-shell columns in comprehensive two-dimensional HPLC: A review. Anal. Bioanal. Chem. 2015; 407: 139-151.
20. Guiochon, G. Monolithic columns in high-performance liquid chromatography. J.Chromatogr. A. 2007; 1168: 101-168.
21. Jandera, P., Hájek, T., Sandra, P. Monolithic and core-shell columns in comprehensive two-dimensional HPLC: A review. Anal. Bioanal. Chem. 2015; 407: 139-151.
22. Lui, J., Regalado, E.L., Mergelsberg, I., Welch, C.J. Extending the range of supercritical fluid chromatography by use of water-rich modifiers. Org. Biomol. Chem. 2013; 11: 4925-4929.
23. Tzananvaras, P.D., Themelis, D.G. Validated high throughput HPLC assay for nimesulide using a short monolithic column. J. Pharm. Biomed. Anal. 2007; 43: 1483-1487.

24. Tzananvaras, P.D., Themelis, D.G. Zotou, A., Stratis, J., Karlsberg, B. Optimization and validation of a dissolution test for selegiline hydrochloride tablets by a novel HPLC assay using a monolithic stationary phase. J. Pharm. Biomed. Anal. 2008; 46: 670-675.
25. Nischang, I., Teasdale, I., Bruggerman, O. Towards porous polymer monoliths for the efficient, retention-independent performance in the isocratic separation of small molecules by means of nano-liquid chromatography. J. Chromatogr. A. 2010; 1217: 7514-7522.
26. Peristyy, A., Fedyanina, O.N., Paull, B., Nesterenko, P.N. Diamond based adsorbents and their applications in chromatography. J.Chromatogr. A. 2014; 1357: 68-86.
27. Peristyy, A., Paull, B., Nesterenko, P. N. Chromatographic performance of synthetic polycrystalline diamond as a stationary phase in normal phase high performance liquid chromatography. J.Chromatogr. A. 2015; 1391: 49-59.
28. Nguyen, D.T.T., Guillarme, D., Rudaz, S., Veuthey, J.L. New trends in fast liquid chromatography. Chimia 2007; 61: 186-189.
29. Heinisch, S., Rocca, J-L. Sense and nonsense of high-temperature liquid chromatography. J. Chromatogr. A. 2009; 1216: 642-658.
30. Al-Othman, Z. A., Al-Warthan, A., Ali, I. Advances in enantiomeric resolution on monolithic chiral stationary phases in liquid chromatography and electro chromatography. J.Sep.Sci. 2014; 37: 1033-1057.
31. Hammon, C., Wong, M., Aliagas, I., Ortwine, D.F., Pease, J., Schmidt, D.E., Victorino, J. The evaluation of 25 chiral stationary phases and the utilization of sub-2μm coated polysaccharide chiral stationary phases via supercritical fluid chromatography. J.Chromatogr. A. 2013; 1305: 310-319.
32. Ai, F., Laisheng, L., Ng, S-C., Tan, T.T.T.Y.: Sub-1-micron mesoporous silica particles functionalized with cyclodextrin derivative for rapid enantioseparations on ultra-high pressure liquid chromatography. J. Chromatogr. A. 2010; 1217:7502-7506.
33. León-González, M. E., Rosales-Conrado, N., Pérez-Arribas, L. V., Guillén-Casla, V. Two-dimensional liquid chromatography for direct chiral separations: a review. Biomed. Chromatogr. 2014; 28: 59-83.
34. Nie, Y., Liu, X., Yang, X., Zhao, Z.: Review: Recent application of chiral liquid chromatography–tandem mass spectrometric methods for enantiomeric pharmaceutical and biomedical determinations. J. Chromatogr. Sci. 2013; 51: 753-763.
35. Tsioupi, D. A., Stefan-vanStaden, R. I., Kapnissi-Christodoulou, C. P. Chiral selectors in CE: Recent developments and applications. Electrophoresis 2013; 34: 178204.
36. Stavrou, I. J., Mavroudi, M. C., Kapnissi-Christodoulou, C. P. Chiral selectors in CE: Recent developments and applications (2012-mid 2014). Electrophoresis 2015; 36: 101-123.
37. Swartz, M.E.. UPLC: An Introduction and Review. J. Liq. Chromatogr. Rel. Technol. 2005; 28: 1253-1263.
38. Chester, T.L.: Sub 2- micron performance with a conventional HPLC instrument using 2.7-micron fused core particles. American Laboratory. 2009; 1-8.
39. Russo, R., Guillarme, D., Nguyen, D.T.T., Bichi, C., Rudaz, S., Veuthey, J.L.: Pharmaceutical applications on columns packed with sub 2μm particles. J. Chromatogr. Sci. 2008; 46: 199-208.
40. Dongre, V.G., Karmuse, P.P., Prakasa Rao, P., Kumar, A.: Development and validation of a UPLC method for the determination of primaquine phosphate and its impurities. J.Pharm. Biomed. Anal. 2008; 46: 236-242.
41. Wren, S.A.C., Tchelitcheff, P.: Use of ultra-performance liquid chromatography in pharmaceutical development. J.Chromatogr. A. 2006; 1119, 140-146.
42. Regalado, E.L., Zhuang, P., Chen, Y., Makarov, A.A., Schafer, W.A., McGachy, N., Welch, C.J. Chromatographic resolution of closely related species in pharmaceutical chemistry: Dehalogenation impurities and mixtures of halogen isomers. Anal. Chem. 2014; 86: 805-813.
43. Ahamed, A., Krishnamurthy, G., Reddy, M., Ramesha, S., Naik, H.S.B., Charitha, J.G. Development and validation of stability indicating ultra-performance liquid chromatographic method for riluzole. J. Pharmacy Res. 2010; 3: 1028-1033.
44. Krishnaiah, C., Vishnumurthy, M., Reddy, A.R., Kumar, R., Mukkanti, K.: Development and validation of ropinirole hydrochloride and its related impurities by UPLC in API and pharmaceutical dosage forms. J. Chinese Chem. Soc. 2014; 57: 348355.
45. Fekete, S., Fekete, J.: The impact of extra-column band broadening on the chromatographic efficiency of 5 cm long narrow-bore very efficient columns. J.Chromatogr. A. 2011; 1218: 5286-5291.
46. Fountain, K.J., Neue, U.D., Grumbach, E.S., Diehl, D.M. Effects of extra-column band spreading, liquid chromatography system operating pressure, and column temperature on the performance of sub-2-μm porous particles. J.Chromatogr. A. 2009; 216: 5979-5988.
47. Periat, A., Boccard, J., Veuthey, J-L., Rudaz, S., Guillarme, D. b. Systematic comparison of sensitivity of hydrophilic interaction chromatography versus reversed phase liquid chromatography and reversed phase liquid chromatography coupled with mass spectrometry. J.Chromatogr. A. 2013; 1312: 49-57.
48. Nguyen, H.P., Schug, K.A.: The advantages of ES-MS detection in conjunction with HILIC mode separations: Fundamentals and applications. J. Sep. Sci. 2008; 31: 1465-1480.
49. Mitchell, C.R., Bao, Y., Benz, N.Y., Zhang, S. Comparison of the sensitivity of evaporative universal detectors and LC/MS in the HILIC and reversed-phase HPLC modes. J.Chromatogr. B. 2009; 877: 4133-4139.
50. Periat, A., Kohler, I., Bugey, A., Bieri, S., Versace, F., Staaub, C., Guillarme, D.: Hydrophilic interaction chromatography versus reversed phase liquid chromatography coupled to mass spectrometry: Effect of electrospray ionization source geometry. J.Chromatogr. A. 2014; 1356: 211-220.
51. Natishan, T.K.: Developments in fast liquid chromatographic analysis of pharmaceuticals. J. Liq. Chromatogr. Rel. Technol. 2011; 34: 1133-1156.
52. Anderson, T., Skuland, I.L., Holm, A., Trones, R., Griebokk, T.: Temperature programmed packed capillary liquid chromatography coupled to evaporative light scattering detection and electrospray ionisation time-of-flight mass spectrometry for characterization of high-molecular-weight hindered amine light stabilities. J. Chromatogr. A. 2004; 1029: 49-56.
53. Edge, A.M., Shillingford, S., Smith, C., Payne, R., Wilson, I.D.: Temperature as a variable in liquid chromatography: Development and application of a model for the separation of model drugs using water as the eluent. J.Chromatogr. A. 2006; 1132: 206-210.
54. Chienthavorn, O., Smith, R., Saha, S., Wilson, I., Wright, B., Taylor, S., Lenz, E.: Superheated water

chromatography-nuclear magnetic resonance spectroscopy and mass spectrometry of vitamins. J.Pharm.Biomed. Anal. 2004; 36: 477-482.

55. Lestremau, F. Cooper, A., Szucs, R., David, K., Sandra, P. New trends in fast and high-resolution liquid chromatography: A critical comparison of existing approaches. J. Chromatogr. A. 2006; 1109: 191-196.
56. Plumb, R., Mazzeo, J.R., Grumbach, E.S., Rainville, P., Jones, M., Wheat, T., Neue, U.B., Smith, B., Jonson, K.A. The application of small porous particles, high temperatures, and high pressures to generate very high resolution LC and LC/MS separations. J. Sep. Sci. 2007; 30:1158-1166.
57. Vanhoenacker, G., Sandra, P. High temperature and temperature programmed HPLC: Possibilities and limitations. Anal. Bioanal. Chem2008; 390:245-248.
58. Lurie, I., Li, L: Use of high temperature liquid chromatography with sub-2µm particle C18 columns for the analysis of seized drugs. J. Liq. Chromatogr. Rel. Technol. 2009; 32: 1255-1260.
59. Albert, M., Cretier, G., Guillarme, D., Heinisch, S., Rocca, J.L.: Some advantages of high temperature for the separation of pharmaceutical compounds with mass spectrometry detection. J. Sep. Sci. 2005; 28: 1803-1811.
60. Heinisch, S., Rocca, J-L.: Sense and nonsense of high-temperature liquid chromatography. J. Chromatogr. A. 2009; 1216, 642-658.

How to cite this article:
Savkare AD, Kalaskar PS, Sarode SK and Potkule ME: Recent advances in impurity profiling of pharmaceuticals. Int J Pharm Sci Res 2017; 8(8):3206-17.doi: 10.13040/IJPSR.0975-8232.8(8).3206-17.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)