Research progress on the impurities identification and determination in pharmaceuticals

wenlong Li[1], Yuru Chen[1], Zhiyong Zhang[1], Xin Gao[1], and Xuting Guo[1]

[1]Tianjin University of Traditional Chinese Medicine

July 10, 2023

Abstract

Rationale: Impurities refer to any substances that affect the purity of pharmaceuticals. Controlling impurities has always been a significant concern during drug development. Impurities impact the drug's purity, diminish the efficacy of pharmaceuticals, and alter their appearance, physical, and chemical properties. Additionally, impurities can compromise the stability of pharmaceuticals and elevate their toxic and side effects. **Methods:** Impurity source analysis is the basis of drug impurity control. To clarify the source of impurity can optimize the synthesis process, prescription process, packaging and storage conditions of the pharmaceuticals, and control the impurity within a reasonable limit to achieve the ultimate goal of impurity research. **Results:** Analysis method is a means to obtain impurity information, and diversified analysis methods are also possible for effective control of different types of impurities. At present, there are relatively many quantitative studies on impurities, but there are still some challenges for the structure analysis of impurities, especially trace impurities. **Conclusion:** The research progress of drug impurity control and evaluation from the sources of various drug impurities and impurity analysis techniques was reviewed in this article, with the aim of providing references for the related research.

Research progress on the impurities identification and determination in pharmaceuticals

Yuru Chen[a#], Zhiyong Zhang[a#], Xin Gao[a], Xuting Guo[a], Wenlong Li[a, b, c]11* Corresponding author. Tel.: +86 22 59596814; Fax: +86 22 59596814.*E-mail address: wshlwl@tjutcm.edu.cn* (W. Li).

[a] College of Pharmaceutical Engineering of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin, 301617, PRC;

[b] State key Laboratory of Component-based Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin, 301617, PRC;

[c] Haihe Laboratory of Modern Chinese Medicine, Tianjin, 301617, PRC

Rationale: Impurities refer to any substances that affect the purity of pharmaceuticals. Controlling impurities has always been a significant concern during drug development. Impurities impact the drug's purity, diminish the efficacy of pharmaceuticals, and alter their appearance, physical, and chemical properties. Additionally, impurities can compromise the stability of pharmaceuticals and elevate their toxic and side effects.

Methods: Impurity source analysis is the basis of drug impurity control. To clarify the source of impurity can optimize the synthesis process, prescription process, packaging and storage conditions of the pharmaceuticals, and control the impurity within a reasonable limit to achieve the ultimate goal of impurity research.

Results: Analysis method is a means to obtain impurity information, and diversified analysis methods are also possible for effective control of different types of impurities. At present, there are relatively many

quantitative studies on impurities, but there are still some challenges for the structure analysis of impurities, especially trace impurities.

Conclusion: The research progress of drug impurity control and evaluation from the sources of various drug impurities and impurity analysis techniques was reviewed in this article, with the aim of providing references for the related research.

Keywords: Impurities in pharmaceuticals; Impurity source identification; Separation and purification techniques; Qualitative and quantitative methods

1 Introduction

As the public pays more and more attention to the safety of drugs, the study of impurities has become the key to drug quality control. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), U.S. Food and Drug Administration (FDA) and other institutions have issued various guidelines for impurities. Among them, the ICH was the first to formulate guidelines on impurities. In 1995 and 1996, guidelines for "Impurities in New Drug Substances" and "Impurities in New Drug Products" were promulgated and revised in subsequent years by the ICH. In the ICH Q3A(R2) guideline, impurities are divided into organic impurities, elemental impurities and residual solvents according to their physical and chemical properties, and the limits of organic impurities are specified based on the permitted daily exposure. If the impurity content is greater than the reporting limit, the impurity needs be reported in the test report. If the impurity content is greater than the identification limit, the chemical structure of the impurity must be confirmed. If the impurity content exceeds the quality control limit, it's necessary to clarify the safety of the impurity, or reduce the impurity content below the quality control limit through process optimization and other ways. [1] Q3C [2]and Q3D [3] also give detailed guidelines for residual solvents and elemental impurities, and they provide guidance on the content and limits of impurity studies for drug declaration. The U.S. FDA also promulgated "Guidance for Industry Drug Product Chemistry, Manufacturing, and Controls Information" in 1998, [4]and provided clear technical requirements for the study of impurities.

Impurities are any substances that affect the purity of drugs. Impurities that are related to the quality of drugs not only affect the purity of medicines and reduce the efficacy, but also affect the safety of human medication. For example, β-lactam antibiotics have the characteristics of strong bactericidal activity and wide indications, but the polymer impurities of β-lactam antibiotics can quickly cause hypersensitivity reactions, especially lactam antibiotics for injection.[5] Chiral impurities that cause adverse reactions are also frequently reported. Li et al. [6] study the toxicity of the chiral compound muscone that is an active ingredient in natural musk. The chiral compound muscone can be divided into s-muscone and r-muscone. The research results show both of them are acutely toxic to zebrafish embryos, but s-muscone has higher toxicity at the same concentration. It's necessary to synthesize the chiral isomer of muscone to ensure the safety of medication. Yi et al. [7]reported the genotoxicity of impurities in the rabeprazole that was used to treat gastrointestinal diseases. The silico analysis that used the Derek and Sarah software showed that the impurity 2-[[4-(3-methoxy propane)-3-methyl -2-pyridyl] methiony l]-1H-benzimidazole has a warning structure, and it is proposed that this impurity should be controlled within the limit of 0.01%. It can be seen that genotoxic impurities are extremely important to the safety of drugs, and some regulations on genotoxic impurities have also been promulgated. The M7 guidelines for genotoxic impurities promulgated by ICH in 2013,[8] "guidline on the limits of genotoxic impurities" promulgated by the European Medicines Agency (EMA) in 2007[9] and the FDA promulgated "Guidance for Industry, Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommend Approaches"in 2008 to put forward clear requirements for genotoxic impurities. [10]

Nowadays, the research concept of impurity has shifted from the concept of purity and limit to the direction of impurity profile. The concept of impurity profile is to understand the source, content, structure, physical and

chemical properties, biological activity of each impurity in the drug, and to give a reasonable quality control limit after integrating all information. Impurity source analysis is the basis of drug impurity control. [11, 12] To clarify the source of impurities has important guiding significance for optimizing the synthesis process, prescription process, packaging and storage conditions of drugs. Analysis method is a instrument to obtain impurity information. Choosing a suitable analysis method can ensure the effective detection of impurities. Diversified analysis methods also make it possible to effectively control different types of impurities. The following introduces the sources of impurities in various drugs, and focuses on the analysis techniques used in the impurity research process.

2 Sources of impurities in various drugs

The ICH guidelines clearly stipulate that the source of impurities in drugs that exceed the identification limit must be clarified. Impurity source analysis is the basis for drug impurity control. Identifying the source of impurities can optimize the synthesis process, prescription process, packaging and storage conditions of the drug. Analyzing the actual impurity generation pathway to optimize drug production, storage, and transpoFrtation by inferring the potential impurities that may be generated, so as to achieve the purpose of impurity control. Fig. 1 shows the main sources of impurities.

2.1 Sources of impurities in synthetic drugs

The impurities of synthetic drugs can be divided into process impurities and degradation products according to their sources. In the production process of synthetic drugs, some starting materials, intermediates and by-products can all be regarded as impurities. [13, 14]Unreasonable prescription process design, reaction between excipients and Active Pharmaceutical Ingredients (API) also produce some impurities. The type and amount of such impurities are often determined by the optimization level of process parameters. Angelo et al.[15] studied the effects of excipients and pH on dimer impurities of the penicillin. Fig. 2 shows the degradation pathways of penicillin. After penicillin 1 is degraded to penicillic acid 2, the pH value of the solution is increased and the degradation of other products is further accelerated. Experiments show that the addition of sodium citrate that was used to change the pH of the solution can inhibit the formation of dimer impurities, but the addition of EDTA have not much effect on the dimer content. Vossen et al. [16]optimized the formulation process of amlodipine liquid formulations that treatment the hypertension in children and adolescents through stability tests to avoid the formation of precipitation during storage.

The oxidation, hydrolysis, polymerization, isomerization and other reactions of API can also introduce new impurities during the storage and transportation of medicines because of various environmental changes, such as: temperature, humidity and light, [17]and unreasonable packaging selection can also cause the polymer or element impurities of the packaging to penetrate into the drug.[18] Such impurities are called degradation products and usually determined the degradation pathway through stability tests and forced degradation tests. [19] The forced degradation test is that the drug is destroyed by high temperature, high humidity, strong light irradiation, acid hydrolysis, alkali hydrolysis and oxidation, [20] which can obtain a large amount of impurity information in a short time and provide guidance for the packaging and storage conditions of drugs to avoid and reduce the generation of drug degradation products.

Some elemental impurities are introduced when the catalysts or reagents added intentionally during the synthesis of drugs. Of course, it is not ruled out that the introduction of elemental impurities is related to contact packaging and metal devices that are not resistant to acid and alkali. [21] The toxicity of elemental impurities cannot be ignored. For example, acute and chronic exposure to cadmium can cause damage to the reproductive system, kidney, liver, bone, lung, cardiovascular and other tissues in the body, and can suppress immunity and cause teratogenesis. [22] Although copper is an essential element for the human body, a high concentration can also cause changes in the body, such as: lipid metabolism, neuronal activity.[23] In addition, the element impurities in the drugs have no effect on curing diseases, and may also catalyze

the degradation of active pharmaceutical ingredients, so as to cause side effects and adverse reactions. The ICH Q3D categorizes element impurities into three categories based on their permitted daily exposure and their likelihood of occurrence in pharmaceuticals and puts forward control requirements for them. [3] Similar regulations were also issued by the current United States Pharmacopoeia (USP) in general chapter <231>, <232> and <233> and European Pharmacopoeia (EP).[24]

2.2 Sources of impurities in natural drugs

The ICH guidelines limit the types of drugs, and only provide a illustrative decision tree for the supervision of impurities in synthetic drugs. Some biopharmaceuticals (vaccines, cell metabolites, plasma, plasma products, etc.) and natural drugs are not applicable. Only some pharmacopoeias provide standards for the quality control of impurities. The natural drugs come from a wide range of sources, and the quality of natural drugs from different places is also quite different. It is likely to be mixed with some improper origins and incorrect medicinal parts to introduce some impurities. Li et al.[25] used Fourier transform near-infrared spectroscopy combined with chemometrics analysis to identify the authenticity of Rhodiola from four different base sources and provide a valuable reference for the safety and effectiveness of clinical application of Rhodiola. In addition, some single-component preparations that extracted and separated from natural drugs will introduce some impurities that similar chemical structures and properties due to insufficient production technology. [26] The natural drugs can absorb or accumulate heavy metal elements from the natural environment during the growth process, and pesticide is used. The impurity inspections of natural drugs preparations also focus on harmful elements,[27] pesticide residues [28] and mycotoxins. [29] Nan et al. [27]discussed the content and proportion of heavy metals Pd, Cu, As, Cd, Hg in different tissues of peony medicinal materials and in different months, and the results showed that the content of different species of heavy metal can change during the growing period of plants and the total content of heavy metals can migrate from different tissues. It is pointed out that it is more scientific and reasonable to analyses the species of heavy metals during growing period of plant medicine.

2.3 Sources of impurities in biopharmaceuticals

Biopharmaceuticals are different from general chemicals. The active ingredients of biopharmaceuticals are generally proteins, peptides, nucleic acids, enzymes, hormones, etc. They are more sensitive to factors such as humidity, temperature, pH, and light. It is prone to degradation changes such as oxidation, aggregation or fission.[30] Biopharmaceuticals will face a complex multi-phase system that contains microbial cells, metabolites, unused culture medium, etc in the production process, so the concentration of the target product is very low and the impurity content is high. It is more difficult to separate the active ingredients and impurities without destroying the activity of the target product. [31] If a drug with higher purity is required, the separation steps required will increase, and the yield of the drug will decrease accordingly. Some new extraction and separation methods have been proposed to solve this challenge. Gaëlle et al. [32] studied the hydrophilic nanohydrogel particles that are used for the extraction and purification of recombinant proteins. Zhang et al. [33] report a bifurcated continuous field-flow fractionation chip for high-yield and high-throughput nucleic acid extraction and purification and increase the nucleic acid extraction rate compared with commercial equipment. The risk of elemental impurities being introduced in biopharmaceuticals is very low. [3] Because the production of biopharmaceuticals does not require metal ions as catalysts or reagents, the elements impurities that added to the culture medium are also trace amounts and will not accumulate, and the purification process of biopharmaceuticals (extraction, separation, etc.) can also remove the introduced elements impurities.

3 Separation methods for impurities

According to the impurities source, it is a big challenge to completely remove the impurities in the medicine. In addition, the analysis method of impurities not only needs detect the known impurities of the drug, but also needs effectively detect the potential impurities in the impurity spectrum concept. Therefore, choosing appropriate analytical methods that accurately distinguish and quantify impurities is a key link in the quality control of drugs. Some detectors can detect impurities without separation. For example, quantitative Nuclear Magnetic Resonance Spectroscopy (qNMR) [34] has been described completely in the qualitative and quantitative methods section. However, it is not feasible for some detectors to directly perform qualitative and quantitative analysis of impurities in drugs. Through separation technology, a single compound can be obtained before detection to ensure the accuracy and authenticity of the detection result. Some separation methods are shown in Table 1.

3.1 Liquid Chromatography

3.1.1 Reversed Phase-High Performance Liquid Chromatography

Nowadays, Reversed Phase-High Performance Liquid Chromatography (RP - HPLC) separation technology is the main method of impurities separation in pharmaceuticals. [35-37] Some new mobile phases and stationary phases have also been studied and applied for the separation of various complex samples. For example, ionic liquids can be used as mobile phase additives to significantly improve the separation effect of components, suppress tailing of chromatographic peaks and shorten analysis time by inhibiting the harmful effect of free residuals of silanol groups in the stationary phase. [38] High temperature liquid chromatography technology can increase the resolution of chromatographic peaks and the analysis speed by increasing the temperature of the chromatographic column. Edgar et al.[39] used high temperature liquid chromatography-tandem mass spectroscopy to determine nine high-intensity sweeteners in a variety of drink samples, and consume only 0.85 m L of a green organic solvent (ethanol). There are also some scholars who introduce quality-by-design principles when they establish analysis methods to obtain the best analysis method, so as to save analysis time and mobile phase consumption. [40]

3.1.2 Hydrophilic Interaction Liquid Chromatography

Hydrophilic Interaction Liquid Chromatography (HILIC), which has a complementary effect with reverse chromatography, but is also different from normal phase chromatography, can retain strongly polar hydrophilic compounds. [41] HILIC does not use a more toxic mobile phase. The characteristic of HILIC is that its stationary phase is bonded with various polar functional groups. [42] Mohit et al. [43] used HILIC - UV to achieve identification and quantification of five potential genotoxic impurities, and compared the separation effect of zorbax silica column, ZIC-HILIC column and nitrile-HILIC column. The zorbax silica column can give the most ideal analysis results. Marta et al. [44] developed an alternative direct chiral HPLC method to separate the (R)-and (S)- ramosetron on a chlorinated cellulose-based chiral stationary phase in HILIC mode. A new method provided to analyze the enantiomer of ramosetron.

3.1.3 Mixed mode column

In order to satisfy the separation of complex samples, some emerging chromatographic columns have been developed and utilized. Mixed mode chromatographic columns combine different fillers and bonded functional groups on the same chromatographic column to separate compounds with large differences. At present, there are many applications of mixed mode chromatography columns in impurity separation. [45-47]Size Exclusion Chromatography (SEC) is an analytical method that realizes separation based on the relative relationship between pore size and molecular weight. [48] It is suitable for the separation of substances with different molecular weights, especially for biological products. With the increase of repeating units, high molecular weight compounds also have great differences in their physical and chemical properties, such as solubility, ionic bonding capacity. When reverse chromatography is used to separate compounds with large molecular weights, these differences may cause precipitation and adsorption, which may cause the chromatographic peak to

broaden and fall below the detection line, or it may not be eluted. [49]Lin et al. [50] proposed a size-exclusion chromatography on a reversed-phase column that separated trace high molecular weight species with a variety of functional groups from high concentration of small molecule substance and solve a majority of high-MW impurity cases in active pharmaceutical ingredient. reversed-phase chromatography-size-exclusion chromatography is the stationary phase of reverse chromatography in the mode of size exclusion chromatography.[51] Because of its high sensitivity and wide solvent compatibility, it can potentially become a method to detect the high molecular weight impurity.

There are many liquid chromatography method for separating impurities in drugs. Haddad's team studied the impact of Ion Chromatography (IC) with universal detectors [52] and organic solvent[53] on the analysis of pharmaceutical impurities. In addition, the application of two-Dimensional Liquid Chromatography[54] (2D-LC) and Ultrahigh Performance Liquid Chromatography [55] (UPLC) facilitates the separation of complex samples and improves the efficiency of impurity analysis. Of course, these impurities separation methods can only be combined with suitable detectors to achieve greater benefits.

3.2 Thin layer chromatography

Thin-layer chromatography (TLC) also plays a key role in the separation and determination of impurities because of economic benefits.[56-58] After the sample is separated, the TLC image can be obtained by a flatbed scanner, and then processed by image analysis software to calculate the area or gray value of the characteristic spots on the TLC thin-layer plate for quantitative analysis. Bagcinele et al.[59] developed a manual Thin Layer Chromatography (TLC) to separate a mixture of all lipopeptide families with other peptide and lipid impurities and validated for the semi quantitative determination of the surfactin lipopeptide family. Precision would be comparable to that of estimation via HPTLC, and the analytical method reduces the measurement cost, time and consumption of organic reagents. Eglal et al.[60] is the first use of TLC method for the determination of aspirin, omeprazole and the impurity of aspirin (salicylic acid). TLC method is eco-friendly and greener when compared to the already reported method.

3.3 Other separation methods

There are many methods for separating impurities in drugs. In the separation of chiral impurities, Supercritical Fluid Chromatography (SFC) is also a good choice. Kalíková et al. [61] used SFC to analyze 21 chiral compounds with different physical and chemical properties on a cellulose 3s-(3,5-dimethylphenylcarbamate)-based chiral stationary phase. It has the best separation effect on β - receptor blockers, and achieve better separation in a shorter time than HPLC. Henrik et al. [62] used Capillary Electrophoresis (CE) method to determine dapoxetine hydrochloride and its enantiomeric impurities and the result was comparable to the data of an enantioselective HPLC method. Enea et al. [63] also gave a detailed review of structurally related compounds in Gas Chromatography (GC) separation of medicines.

4 Qualitative and quantitative methods of impurities

The original quantification of impurities mainly relied on chemical methods, such as volumetric method. So far, some scholars have used this method to determine impurities in drugs. [64] With the rapid development of various detection methods, the combined of chromatography and various detectors has become the main means of impurity analysis today. Quantitative methods for impurities in many pharmacopoeias can be divided into an external standard method with impurity reference standards, the principle component self-control method with correction factors, and the principle component self-control method without correction factors. [65] The most ideal quantitative method is an external standard method with impurity reference standards, but it is difficult to obtain impurity reference standards in the early stage of impurity research, so this method has certain limitations on the quantitative research of impurities. The principle component self-control method without correction factors also has many shortcomings. When the UV detector is used to

quantify unknown impurities, the response of UV detectors for impurities and sample are also different, due to the difference of chemical structure between impurities and impurities, and between impurities and samples. Even the impurities do not have UV absorption. Some mass-type detectors can reduce the response difference between compounds when using the principle component self-control method without correction factors, and they are also widely used in the detection of impurities. Such as Evaporative Light Scattering Detector (ELSD), [66]Charged Aerosol Detector (CAD), [67] Refractive Index (RI), [68] Chemical Lumines-cent Nitrogen-specific Detector (CLND). [69] Although the quantitative capabilities of these detectors are strong, their qualitative capabilities are weak, especially for the direct qualitative determination of unknown substances, which requires the use of known reference materials or related chromatographic qualitative reference data (retention time) for qualitative identification. This also highlights the advantages of Mass Spectrometer (MS) and Nuclear Magnetic Resonance spectrometer (NMR) in impurity qualitative analysis. Fig. 3 shows the general process of impurity characterization.

4.1 Mass Spectroscopy

Because the combination of MS and various chromatograms integrates the high separation capability of chromatography with the high sensitivity of mass spectroscopy, Liquid Chromatography-Mass Spectroscopy (LC-MS) has become the preferred technique for drug impurity analysis. The advantages of MS for impurity analysis are the identification of known impurities and the structure derivation of unknown impurities. The application of high-resolution mass spectroscopy can not only distinguish compounds with very similar molecular weights, but also determine the elemental composition of impurities. After that, the general chemical structure of the unknown compound can be determined by deriving the fragmentation patterns of impurities. Zhu et al.[70] used LC-MS to study the fragmentation patterns of impurities in sodium drug substance and eye drops, deduced the chemical structures of impurities, confirmed the structure with 1D and 2D NMR data, and achieved the structures characterization of 2 unknown impurities and 6 unknown degradation products and a plausible mechanism for the formation of the degradation products was also proposed. Hertzler et al. [71] study fragmentation patterns and fragmentation pathways of paromomycin impurities based on UHPLC/MS/MS and the literature of other structurally related aminoglycoside compounds, and made reasonable suggestions for the storage methods of drugs.

A summary of the fragmentation patterns of similar drugs is helpful to quickly analyze the structure of impurities in the drug. However, this method is only an auxiliary function for structure identification, and it is impossible to sure the position and spatial configuration of some groups in the structure. NMR is required to further characterize the impurities. [72] Mass spectroscopy fragment information can be clarified through many databases, [73] but there is no comprehensive mass spectroscopy fragment database for impurity research.

The difference between MS and other detectors is not only in the structural analysis of impurities, but also in that it can detect very small amounts of impurities, which greatly improves the safety of drugs containing low levels of highly toxic impurities. Isotope internal standard and multiple reaction monitoring (MRM) mode are unique features of mass spectroscopy quantification. Isotope as an internal standard quantification method can reduce the accidental errors caused by separate injection of standards, [74] but this method has certain limitations because the standard of impurities is not easy to obtain, and it is mostly used to determine known impurities, such as mycotoxins. [75] The MRM mode can realize the simultaneous, exclusive, sensitive and rapid quantitative detection of dozens of similar impurities with different concentration levels by detecting specific ions of the target compound. [76] It is very conducive to the simultaneous quantitative determination of low concentration and multiple structurally similar impurities in complex systems. [77]

In response to the problem that the mobile phase for LC - MS can not contain non-volatile salts, so that many chromatographic methods cannot be directly converted to LC-MS methods. Some researchers also use column switching technology to introduce the mobile phase containing non-volatile salts into the desalted chromatographic column through an on-off valve and then enter the MS for analysis. [78] In the case of low response impurity, deriving and adding alkali metals solve this problem. Wijk et al. [79] chose 1-(pyridin-4-yl)

piperidine 4-carboxylate (BPPC) as a new, selective pre-column derivatization reagent to obtain reagent related fragmentation of the whole reagent as well as a side group of the reagent when analyzing potential genotoxic compounds.

MS detectors are also commonly used for the detection of elemental impurities. Inductively coupled plasma-mass spectroscopy (ICP - MS) can analyze almost all elemental impurities. ICP - MS has the advantages of fast determination speed, low detection limit, wide detection range, and simultaneous determination of multiple elements. [80]Zheng et al. [81] established an ICP - MS method to determinate 24 elemental impurities of the ICH guidelines in ubenimex API after direct dissolution in diluted acid solution and successfully applied to the elemental impurities determination in 3 batches of ubenimex API from different factories. There are many analytical methods for impurity elements. Such as colorimetry, Flame Absorption Spectroscopy (FAAS), [82] Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP - AES), [83]X-Ray Fluorescence Spectroscopy (XRFS), [84] and Atomic Fluorescence Spectrometry (AFS). Their advantages and disadvantages are shown in Table 2. In contrast, ICP - MS relies on the advantages of the MS detector are more suitable for trace and ultra-trace element analysis.

4.2 Nuclear Magnetic Resonance spectroscopy

Nuclear magnetic resonance spectroscopy technology can play an important role in the analysis of impurities in drugs, not only qualitatively or quantitatively. MS and its combined technology can deduce the structure of impurities, but it can not obtain the exact chemical structure of impurities, while NMR can provide comprehensive structural information (planar structure, relative structure, three-dimensional structure) and is also an accepted method to determine the identification structure of organic compounds. If NMR analyzes the structure of a compound, the sample needs to reach the mg level and the purity of the sample is very high. However, the sensitivity of NMR is low, which is also a huge challenge to the structure analysis of impurities. There are also some researchers who use preparative HPLC to enrich the impurities in the samples, so that the amount of impurities reaches the detection line of NMR. [85-87] However, the amount of impurities in the sample is very low. If it is directly extracted and separated from the medicine, the preparation is difficult and the cost is high. Some researchers increase the content of impurities in the sample through forced degradation experiments to reduce the workload of preparation.[88] There are also researchers who obtain a large amount of impurities through synthesis methods, [89-91]but this method needs to derive the structure of the impurities, then design the synthesis route, and finally verify the synthesized impurities. It has many steps, takes a long time, and the success rate is not high.

Follow the idea of LC - MS. The combination of liquid phase and NMR will also become a possibility. However, the hyphenation of LC - NMR has a little limitation on impurity analysis, because the accumulation time of each chromatographic peak is too short to obtain NMR spectra of minor impurities and the stopped-flow technique leading to peak diffusion in the column during accumulation. [92] LC - NMR analysis can be conducted with a cryogenic probe to cool the radiofrequency coil and preamplifier, leading to a reduction in the thermal noise and an increase in the detection sensitivity. [93] In addition, ultra-high field magnets (> 800 MHz) can be used in LC-NMR to improve the detection sensitivity, but it has been little progress over the past decade. [94] Takashi et al.[95] constructed a UHPLC - NMR system to concentrate chromatographic peaks. In the UHPLC - NMR system, the magnetic field strength was increased, a cryogenic probe was used to improve the sensitivity, and the loop-storage technique was used to suppress diffusion. The schematic diagram of UHPLC-NMR system is shown in Fig. 4. The sensitivity is higher than an ultra-high field magnet (800 MHz) and a probe.

In the early stage of drug research, it is often difficult to have standards for impurities, especially unknown impurities, but NMR can accurately quantify impurities without reference materials. The qNMR quantitative technology is based on the fact that the area of the nuclear magnetic resonance spectrum signal is proportional to the number of excited atoms in the sample to achieve the quantitative goal.[96] The qNMR quantitative method is divided into external standard method and internal standard method. The result of external standard method is greatly affected by the instrument, so the internal standard method is usu-

ally used for quantification.[97] Commonly used internal standards are benzoic acid, maleic acid and fumaric acid. The structure and content of the internal standard should be known, and the response signal of the internal standard should be well separated from the response signal of the impurity to be measured without overlapping. Naoki et al.[98] also propose a novel extended internal standard method of qNMR assisted by chromatography (EIC) that accurately quantifies 1H signal areas of samples, when the chemical shifts of the impurity and samples signals overlap completely, and used 2-chlorophenol and 4-chlorophenol containing phenol as an impurity as examples in which impurity and samples signals overlap to validate and demonstrate the method. Liu et al. [99] used q NMR and HPLC - UV to determine ten impurities of cefazolin and provided the relative response factors of ten impurities. Made up for the defect the principle component self-control method without correction factors in the determination of related substances. Compared with LC - UV that commonly used for impurity analysis, qNMR does not require complicated pre-separation processing. For the determination of drug content without ultraviolet absorption in molecular structure, or corresponding reference substance, qNMR is also a very suitable method.[100]

5 Conclusions

At present, there are relatively many quantitative studies on impurities, but there are still some challenges for the structure analysis of impurities, especially trace impurities. The ICH guidelines clearly point out that it is necessary to analyze the structure of impurities exceeding a certain content in the quality control of drugs. Although the sensitivity of MS can reach the microgram level, there are still some problems in the identification of unknown impurity structures. Because MS only deduces the approximate chemical structure based on the fragmentation law. The structural characterization of impurities also needs to rely on more spectral information, such as NMR, infrared spectroscopy. However, the sensitivity of NMR spectroscopy is poor, and the structural characterization of trace impurities is still a huge challenge. Due to the small amount of impurities in the sample, it is not easy to obtain a large amount of impurities by preparative HPLC. The impurity can be enriched by forced degradation and directed synthesis, so that the impurities in the sample can reach the detection line and purity, but this method does not solve all the impurity problems. Improving the sensitivity of NMR and the system of LC - NMR may be solutions to the detection of trace impurities, and it is also the direction that many researchers are working on.

In many cases, the quantification of unknown impurities lacks standard products, so that absolute quantification of impurities cannot be achieved. Some researchers have also established a method of impurity reference substance database [101] to solve this problem. The database provides qualitative parameters of impurity (chromatographic retention time, UV/IR, MS and NMR) and quantitative parameters of impurity (principal component response factors). It is also a good choice to solve the accurate quantification of impurities.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are extremely grateful to the financial support of Tianjin Science and technology project (No. 20ZYJDJC00090).

References

1. ICH. (2006) Q3A(R2): Impurities: Impurities in New Drug Substances. (accessed December 12, 2021).
2. ICH. (2021) Q3C(R8): Impurities: Guideline for Residual Solvents. (accessed December 12, 2021).
3. ICH. (2022) Q3D(R2): Impurities: Guideline for elemental impurities. (accessed April 26, 2022).

4. Dhangar KR, Jagtap RB, Surana SJ, et al. Impurity profiling of drugs towards safety and efficacy: theory and practice. *J Chil Chem Soc.* 2017;62(2): 3543-3557.*doi.org/10.4067/S0717-97072017000200024* .
5. Xu Y, Wang DD, Tang L, et al. Separation and characterization of allergic polymerized impurities in cephalosporins by 2D-HPSEC× LC-IT-TOF MS. *J Pharm Biomed Anal* . 2017;145: 742-750. doi.org/10.1016/j.jpba.2017.07.063
6. Du Y, Wu Y, Liu Y, et al. Identification and genotoxicity evaluation of potential impurities in rabeprazole sodium using in silico and in vitro analyses. Drug Chem Toxicol. 2022; 45(5): 2116-2122. doi.org/10.1080/01480545.2021.1908712
7. Li M, Yao L, Chen H, et al. Chiral toxicity of muscone to embryonic zebrafish heart. *Aquat Toxicol* . 2020; 222: 105451. doi.org/10.1016/j.aquatox.2020.105451
8. ICH (2017) M7(R1): Mutagenic impurities: Assessment And Control of DNA reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, (accessed December 12, 2021).
9. Snodin DJ. Elusive impurities—evidence versus hypothesis. Technical and regulatory update on alkyl sulfonates in sulfonic acid salts. Org Process Res Dev. 2019; 23(5): 695-710. doi.org/10.1021/acs.oprd.8b00397
10. U. S. FDA. 2008. Guidance for Industry, Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommend Approaches.
11. Gogna K. Regulatory aspects of impurity profiling. International Journal of Drug Regulatory Affairs, 2020; 8(4): 45-54. doi.org/10.22270/ijdra.v8i4.433
12. Yue Y, Wang J, Zhao Y, et al. Impurity profiling of Cefteram pivoxil based on Fourier transform ion cyclotron resonance MS. *J Pharm Biomed Anal* . 2020; 191: 113591. doi.org/10.1016/j.jpba.2020.113591
13. Chen Y, Wu S, Yang Q. Development and validation of LC-MS/MS for analyzing potential genotoxic impurities in Pantoprazole starting materials. *J Anal Methods Chem* . 2020; 2020. doi.org/10.1155/2020/6597363
14. Martin S, Tonnesmann R, Hierlmeier I, et al. Identification, characterization, and suppression of side products formed during the synthesis of [177Lu] Lu-PSMA-617. *J Med Chem* . 2021; 64(8): 4960-4971. doi.org/10.1021/acs.jmedchem.1c00045
15. Viola A, Ferrazzano L, Martelli G, et al. Novel insights into the chemistry of an old medicine: a general degradative pathway for penicillins from a piperacillin/tazobactam stability study. *Eur J Pharm Sci* . 2019; 136: 104957. doi.org/10.1016/j.ejps.2019.104957
16. Van der Vossen AC, Van Der Velde I, Smeets O, et al. Design and stability study of an oral solution of amlodipine besylate for pediatric patients. *Eur J Pharm Sci* . 2016; 92: 220-223. doi.org/10.1016/j.ejps.2016.05.019
17. Arruda C, Ribeiro VP, Almeida MO, et al. Effect of light, oxygen and temperature on the stability of artepillin C and p-coumaric acid from Brazilian green propolis. *J Pharm Biomed Anal* . 2020; 178: 112922. doi.org/10.1016/j.jpba.2019.112922
18. Singh G, Gollapalli R, Blinder A, et al. Identification of leachable impurities in an ophthalmic drug product originating from a polymer additive Irganox 1010 using mass spectroscopy. J Pharm Biomed Anal. 2018; 152: 197-203. doi.org/10.1016/j.jpba.2018.01.053
19. Prajapati PB, Bhayani DR, Mehta PJ. Accelerated stability testing of levosimendan: isolation and characterization of degradation impurities utilizing preparative HPLC, LC–MS, NMR, and IR. J Liq Chromatogr Relat Technol. 2018; 41(9): 498-507. doi.org/10.1080/10826076.2018.1470982
20. Mahajan AA, Marathe AM, Jarande SS, et al. Characterization and toxicity evaluation of degradation products of febantel. *Future Journal of Pharmaceutical Sciences.* 2020; 6: 1-12. doi.org/10.1186/s43094-020-00138-7
21. Paskiet D, Kraft C, Tullo E, et al. Assessment of Extractable Elements from Elastomers. *PDA J Pharm Sci Technol* . 2019; 73(1): 83-91. doi.org/10.5731/pdajpst.2017.008193
22. Johri N, Jacquillet G, Unwin R. Heavy metal poisoning: the effects of cadmium on the kidney. BioMetals. 2010; 23: 783-792. doi.org/10.1007/s10534-010-9328-y
23. Gaetke LM, Chow-Johnson HS, Chow CK. Copper: toxicological relevance and mechanisms. Arch

Toxicol. 2014; 88: 1929-1938. doi.org/10.1007/s00204-014-1355-y
24. Chahrour O, Malone J, Collins M, et al. Development and validation of an ICP-MS method for the determination of elemental impurities in TP-6076 active pharmaceutical ingredient (API) according to USP< 232>/< 233. J Pharm Biomed Anal. 2017; 145: 84-90. doi.org/10.1016/j.jpba.2017.06.045
25. Morley R, Minceva M. Trapping multiple dual mode liquid-liquid chromatography: Preparative separation of nootkatone from a natural product extract. *J Chromatogr A* . 2020; 1625: 461272. doi.org/10.1016/j.chroma.2020.461272
26. Li T, Su C. Authenticity identification and classification of Rhodiola species in traditional Tibetan medicine based on Fourier transform near-infrared spectroscopy and chemometrics analysis. Spectrochim. Acta, Part A. 2018; 204: 131-140. doi.org/10.1016/j.saa.2018.06.004
27. Nan G, Guo L, Gao Y, et al. Speciation analysis and dynamic absorption characteristics of heavy metals and deleterious element during growing period of Chinese peony. Int J Phytorem. 2019; 21(14): 1407-1414. doi.org/10.1080/15226514.2019.1633261
28. Cao P, Wang G, Wei X, et al. How to improve CHMs quality: Enlighten from CHMs ecological cultivation. Chin Herb Med. 2021; 13(3): 301-312. doi.org/10.1016/j.chmed.2021.04.014
29. Bi B, Bao J, Xi G, et al. Determination of multiple mycotoxin residues in Panax ginseng using simultaneous UPLC-ESI-MS/MS. J Food Saf. 2018; 38(4): e12458. doi.org/10.1111/jfs.12458
30. Lee MJ, Ramanathan S, Ismail R, et al. Stability test for the enzyme immunoassay reagents of Mitragynine. Asian Pac J Trop Dis. 2014; 4(3): 244. doi.org/10.1016/S2222-1808(14)60553-3
31. Luo H, Li Y, Robbins D, et al. Safety risk management for low molecular weight process-related impurities in monoclonal antibody therapeutics: Categorization, risk assessment, testing strategy, and process development with leveraging clearance potential. Biotechnol Prog. 2021; 37(3): e3119. doi.org/10.1002/btpr.3119
32. Zhang C, Sun G, Senapati S, et al. A bifurcated continuous field-flow fractionation (BCFFF) chip for high-yield and high-throughput nucleic acid extraction and purification. Lab Chip. 2019; 19(22): 3853-3861. doi.org/10.1039/C9LC00818G
33. Levourch G, Lebaz N, Elaissari A. Hydrophilic submicron nanogel particles for specific recombinant proteins extraction and purification. Polymers. 2020; 12(6): 1413. doi.org/10.3390/polym12061413
34. Bradley SA, Jackson Jr WC, Mahoney PP. Measuring Protein Concentration by Diffusion-Filtered Quantitative Nuclear Magnetic Resonance Spectroscopy. Anal Chem. 2019; 91(3): 1962-1967. doi.org/10.1021/acs.analchem.8b04283
35. Beg S, Malik AK, Afzal O, et al. Systematic development and validation of a RP-HPLC method for estimation of abiraterone acetate and its degradation products. J Chromatogr Sci. 2021; 59(1): 79-87. doi.org/10.1093/chromsci/bmaa080
36. Palacharla SK, Krishna Mohan GV, Naga Babu A. RP-HPLC estimation of bumetanide and its impurities in oral solid dosage form. Asian J. Chem. 2019; 31(10): 2275-2283. Doi: 10.14233/ajchem.2019.22069
37. Luo L, Tan M, Luo Y. Determination of related substances in ketoprofen injection by RP-HPLC method. Pak J Pharm Sci. 2019; 32(4): 1607-1615.
38. Mieszkowski D, Koba M, Marszałł MP. Application of Ionic Liquids for the Determination of Lipophilicity Parameters Using TLC Method, and QSRR Analysis for the Antipsychotic Drugs. Med chemistry. 2020; 16(7): 848-859. doi.org/10.2174/1573406415666190723162959
39. Ordoñez EY, Rodil R, Quintana JB, et al. Determination of artificial sweeteners in beverages with green mobile phases and high temperature liquid chromatography–tandem mass spectrometry. Food chemistry. 2015; 169: 162-168. doi.org/10.1016/j.foodchem.2014.07.132
40. Mohan TSSJ, Jogia HA, Mukkanti K. A stability indicating UHPLC method for the simultaneous estimation of perindopril, indapamide in presence of potential impurities: An application of QbD for robustness study. Anal Chem Lett. 2020; 10(4): 477-497. doi.org/10.1080/22297928.2020.1817776
41. Kasagić-Vujanović I, Jančić-Stojanović B. Quality by Design oriented development of hydrophilic interaction liquid chromatography method for the analysis of amitriptyline and its impurities. J Pharm Biomed Anal. 2019; 173: 86-95. doi.org/10.1016/j.jpba.2019.05.026

42. Douša M. Quantification of 2-aminoisobutyric acid impurity in enzalutamide bulk drug substance using hydrophilic interaction chromatography with fluorescence detection. J Pharm Biomed Anal. 2019; 164: 296-301. doi.org/10.1016/j.jpba.2018.10.049
43. Jain M, Srivastava V, Kumar R, et al. Determination of five potential genotoxic impurities in dalfampridine using liquid chromatography. J Pharm Biomed Anal. 2017; 133: 27-31. doi.org/10.1016/j.jpba.2016.10.013
44. Colombo M, Ferretti R, Zanitti L, et al. Direct separation of the enantiomers of ramosetron on a chlorinated cellulose-based chiral stationary phase in hydrophilic interaction liquid chromatography mode. J Pharm Biomed Anal. 2020; 43(13): 2589-2593. doi.org/10.1002/jssc.202000290
45. Kühnreich R, Holzgrabe U. Impurity profiling of l-methionine by HPLC on a mixed mode column. J Pharm Biomed Anal. 2016; 122: 118-125. doi.org/10.1016/j.jpba.2016.01.057
46. Chin S, Lin XX, Santarra B, et al. Multiplexed small molecule impurity monitoring in antibody-based therapeutics by mixed-mode chromatography paired with charged aerosol detection. J Pharm Biomed Anal. 2021; 197: 113952. doi.org/10.1016/j.jpba.2021.113952
47. García-Gómez D, Díaz B A, Rodríguez-Gonzalo E. LC-HRMS based on mixed-mode chromatography for the separation of teicoplanin and the unravelment of its composition. J Pharm Biomed Anal. 2020; 186: 113308. doi.org/10.1016/j.jpba.2020.113308
48. Chen D, Yuan Y, Yu J, et al. Purification of semiconducting polymer dots by size exclusion chromatography prior to cytotoxicity assay and stem cell labeling. Anal Chem. 2018; 90(9): 5569-5575. Doi:10.1021/acs.analchem.8b00095
49. Glöckner G, van der Berg JHM. Precipitation and adsorption phenomena in polymer chromatography. J Chromatogr A. 1986; 352: 511-522. doi.org/10.1016/S0021-9673(01)83405-1
50. Lin Z, Yun KY, Ling M, et al. High-molecular weight impurity screening by size-exclusion chromatography on a reversed-phase column. J Pharm Biomed Anal. 2021; 196: 113908. doi.org/10.1016/j.jpba.2021.113908
51. Xu Y, Wang D, Zhu B, et al. Separation and characterization of allergenic polymerized impurities from cephalosporin for injection by trap free two-dimensional high performance size exclusion chromatography× reversed phase liquid chromatography coupled with ion trap time-of-flight mass spectrometry. J Pharm Biomed Anal. 2018; 154: 425-432. doi.org/10.1016/j.jpba.2018.03.043
52. Karu N, Hutchinson JP, Dicinoski GW, et al. Determination of pharmaceutically related compounds by suppressed ion chromatography: IV. Interfacing ion chromatography with universal detectors. J Chromatogr A. 2012; 1253: 44-51. doi.org/10.1016/j.chroma.2012.06.101
53. Karu N, Dicinoski GW, Hanna-Brown M, et al. Determination of pharmaceutically related compounds by suppressed ion chromatography: I. Effects of organic solvent on suppressor performance. J Chromatogr A. 2011; 1218(50): 9037-9045. doi.org/10.1016/j.chroma.2011.10.011
54. Xu F, Xu Y, Liu G, et al. Separation of twelve posaconazole related stereoisomers by multiple heart-cutting chiral–chiral two-dimensional liquid chromatography. J Chromatogr A. 2020; 1618: 460845. doi.org/10.1016/j.chroma.2019.460845
55. Ahmad I AH, Chen W, Halsey HM, et al. Multi-column ultra-high performance liquid chromatography screening with chaotropic agents and computer-assisted separation modeling enables process development of new drug substances. Analyst, 2019; 144(9): 2872-2880. doi.org/10.1039/C8AN02499E
56. Miniyar PB, Kulkarni RD, Thomas AB, et al. Development and validation of an analytical method for the identification of 2-nitrophenyl (phenyl) sulfane as potential genotoxic impurity of quetiapine fumarate at trace levels by high-performance thin-layer chromatography. JPC-Journal of Planar Chromatography-Modern TLC. 2019; 32(6): 511-516. doi.org/10.1556/1006.2019.32.6.10
57. Soliman SM. Factor optimization study to develop and validate a reversed-phase thin-layer chromatography method for the determination of trimetazidine dihydrochloride and its reported impurities in pharmaceuticals. JPC-Journal of Planar Chromatography-Modern TLC. 2019; 32(4): 273-283. doi.org/10.1556/1006.2019.32.4.2
58. Abdelaleem EA, Naguib IA, Farag SA, et al. Reversed-phase high-performance liquid chromatography and high-performance thin-layer liquid chromatography methods for simultaneous determination of

theophylline, Guaifenesin and guaifenesin impurity (Guaiacol) in their bulk powders and in dosage form. J Chromatogr Sci. 2018; 56(9): 846-852. doi.org/10.1093/chromsci/bmy062

59. Dlamini B, Rangarajan V, Clarke KG. A simple thin layer chromatography based method for the quantitative analysis of biosurfactant surfactin vis-a-vis the presence of lipid and protein impurities in the processing liquid. Biocatal Agric Biotechnol. 2020; 25: 101587. doi.org/10.1016/j.bcab.2020.101587
60. Abdelaleem EA, Abou El Ella DA, Mahmoud AM, et al. Green analysis of newly approved binary omeprazole/aspirin mixture in presence of aspirin impurity using ultra-high-performance liquid chromatography and thin-layer chromatography methods. Biomed Chromatogr. 2021; 35(2): e4986. doi.org/10.1002/bmc.4986
61. Kalikova K, Martinkova M, Schmid MG, et al. Cellulose tris-(3, 5-dimethylphenylcarbamate)-based chiral stationary phase for the enantioseparation of drugs in supercritical fluid chromatography: comparison with HPLC. J Sep Sci. 2018; 41(6): 1471-1478. doi.org/10.1002/jssc.201701341
62. Harnisch H, Scriba GKE. Capillary electrophoresis method for the determination of (R)-dapoxetine,(3S)-3-(dimethylamino)-3-phenyl-1-propanol,(S)-3-amino-3-phenyl-1-propanol and 1-naphthol as impurities of dapoxetine hydrochloride. J Pharm Biomed Anal. 2019; 162: 257-263. doi.org/10.1016/j.jpba.2018.09.039
63. Pagliano E, Campanella B, D'Ulivo A, et al. Derivatization chemistries for the determination of inorganic anions and structurally related compounds by gas chromatography-a review. Anal Chim Acta. 2018; 1025: 12-40. doi.org/10.1016/j.aca.2018.03.043
64. Liu S, Yao S, Zhang H, et al. Determination of relative response factors of cefazolin impurities by quantitative NMR. AAPS PharmSciTech. 2017; 18: 1895-1900. doi.org/10.1208/s12249-016-0654-4
65. Villedieu-Percheron E, Ferreira V, Campos JF, et al. Quantitative determination of Andrographolide and related compounds in Andrographis paniculata extracts and biological evaluation of their Anti-Inflammatory Activity. Foods. 2019; 8(12): 683. doi.org/10.3390/foods8120683
66. Pawellek R, Schilling K, Holzgrabe U. Impurity profiling of l-aspartic acid and glycine using high-performance liquid chromatography coupled with charged aerosol and ultraviolet detection. J Pharm Biomed Anal. 2020; 183: 113149. doi.org/10.1016/j.jpba.2020.113149
67. Katakam LNR, Dongala T. A novel RP-HPLC refractive index detector method development and validation for determination of trace-level alcohols (un-sulfated) in sodium lauryl sulfate raw material. Biomed Chromatogr. 2020, 34(7): e4827. doi.org/10.1002/bmc.4827
68. Wang C, Chen S, Caceres-Cortes J, et al. Chromatography-based methods for determining molar extinction coefficients of cytotoxic payload drugs and drug antibody ratios of antibody drug conjugates. J Chromatogr A. 2016; 1455: 133-139. doi.org/10.1016/j.chroma.2016.05.086
69. Kumar SA, Bhaskar BL. Spectroscopic and volumetric techniques for the estimation of Ivabradine impurity 3, 3'-(propane-1, 3-diyl) bis (7, 8-dimethoxy-1, 3, 4, 5-tetrahydro-2H-benzo [d] azepin-2-one). Int J Appl Sci. 2019; 11(3): 216-218.
70. Zhu P, Lu J, Wang Z, et al. Characterization of impurities in sodium cromoglycate drug substance and eye drops using LC-ESI-ion trap MS and LC-ESI-QTOF MS. J Pharm Biomed Anal. 2017; 145: 537-548. doi.org/10.1016/j.jpba.2017.07.015
71. Hertzler SA, Knuth K, Preston R, et al. Investigation of unknown impurities of paromomycin in a 15% topical cream by liquid chromatography combined with mass spectrometry. Rapid Commun Mass Spectrom. 2019; 33(21): 1660-1669. doi.org/10.1002/rcm.8513
72. Patil S, Kantikar G, Koppula S, et al. Identification and characterization of a new process related impurity in terbutaline sulfate by Accurate-Mass Q-TOF LC/MS/MS and NMR. Chromatographia. 2021; 84: 381-391. doi.org/10.1007/s10337-021-04021-2
73. Pesek M, Juvan A, Jakos J, et al. Database Independent Automated Structure Elucidation of Organic Molecules Based on IR, 1H NMR, 13C NMR, and MS Data. J Chem Inf Model. 2020; 61(2): 756-763. doi.org/10.1021/acs.jcim.0c01332
74. Pagliano E, Meija J. A tool to evaluate nonlinearity in calibration curves involving isotopic internal standards in mass spectrometry. Int J Mass Spectrom. 2021; 464: 116557. doi.org/10.1016/j.ijms.2021.116557

75. Andrade PD, Dantas RR, de Moura TLS, et al. Determination of multi-mycotoxins in cereals and of total fumonisins in maize products using isotope labeled internal standard and liquid chromatography/tandem mass spectrometry with positive ionization. J Chromatogr A. 2017; 1490: 138-147. doi.org/10.1016/j.chroma.2017.02.027
76. Jin B, Guo K, Zhang T, et al. Simultaneous determination of 15 sulfonate ester impurities in phentolamine mesylate, amlodipine besylate, and tosufloxacin tosylate by LC-APCI-MS/MS. J Anal Methods Chem. 2019; 2019. doi.org/10.1155/2019/4059765
77. Babu MD, Babu SK, Kishore K. Development and validation of a GC-MS with SIM method for the determination of trace levels of methane sulfonyl chloride as an impurity in Itraconazole API. J Anal Bioanal Tech. 2016; 7(2): 10.4172. DOI: 10.4172/2155-9872.1000316
78. Iliou K, Malenović A, Loukas Y L, et al. Analysis of potential genotoxic impurities in rabeprazole active pharmaceutical ingredient via Liquid Chromatography-tandem Mass Spectrometry, following quality-by-design principles for method development. J Pharm Biomed Anal. 2018; 149: 410-418. doi.org/10.1016/j.jpba.2017.11.037
79. Van Wijk AM, Niederländer HAG, Siebum AHG, et al. A new derivatization reagent for LC–MS/MS screening of potential genotoxic alkylation compounds. J Pharm Biomed Anal. 2013; 74: 133-140. doi.org/10.1016/j.jpba.2012.10.004
80. Zhao MJ, Cheng L, Huang YJ, et al. Establishment and Validation of an ICP-MS Method for Simultaneous Measurement of 24 Elemental Impurities in Ubenimex APIs According to USP/ICH guidelines. Curr Pharm Anal. 2021; 17(6): 723-730. doi.org/10.2174/1573412916999200423103711
81. Jurowski K, Krośniak M, Fołta M, et al. The toxicological analysis of Cu, Mn and Zn as elemental impurities in pharmaceutical herbal products for teething available in pharmacies in Poland. J Trace Elem Med Biol. 2019; 53: 109-112. doi.org/10.1016/j.jtemb.2019.02.011
82. Janchevska K, Stafilov T, Memed-Sejfulah S, et al. ICH Q3D based elemental impurities study in liquid pharmaceutical dosage form with high daily intake–comparative analysis by ICP-OES and ICP-MS. Drug Dev Ind Pharm. 2020; 46(3): 456-461. doi.org/10.1080/03639045.2020.1724136
83. Sauer B, Xiao Y, Zoontjes M, et al. Application of X-ray fluorescence spectrometry for screening pharmaceutical products for Elemental Impurities according to ICH guideline Q3D. J Pharm Biomed Anal. 2020; 179: 113005. doi.org/10.1016/j.jpba.2019.113005
84. Katakam LNR, Aboul-Enein HY. Elemental impurities determination by ICP-AES/ICP-MS: A review of theory, interpretation of concentration limits, analytical method development challenges and validation criterion for pharmaceutical dosage forms. Curr Pharm Anal. 2020; 16(4): 392-403. doi.org/10.2174/1573412915666190225160512
85. Zhang Q, Cheng Y, Yang J, et al. Isolation, identification, and characterization of potential impurities of doramectin and evaluation of their insecticidal activity. J Pharm Biomed Anal. 2020; 191: 113600. doi.org/10.1016/j.jpba.2020.113600
86. Wang J, Zhou J, Xu Y, et al. Characterization of two unknown impurities in roxithromycin by 2D LC–QTOF/MS/MS and NMR. J Pharm Biomed Anal. 2020; 184: 113196. doi.org/10.1016/j.jpba.2020.113196
87. Narkedimilli J, Vavilala V, Mohanty S, et al. Isolation and structure characterization of related impurity in olanzapine key starting material by LC/ESI-MS and NMR. Asian J Res Chem. 2018; 11(3): 539-544. doi.org/10.5958/0974-4150.2018.00096.2
88. Santhosh, G , Anji Karun Mutha, et al. Isolation and Structural Characterization of Degradation Products of Finasteride by Preparative HPLC, HRMS and 2D NMR; Asian J Chem. 2019; 31: 1514-1518. doi:10.14233/ajchem.2019.21955
89. Rajesh Reddy P, Musunuri S, Rama Sekhara Reddy D, et al. Identification, synthesis, and characterization of potential genotoxic impurities of sildenafil citrate drug substance. Future Journal of Pharmaceutical Sciences. 2020; 6: 1-10. doi.org/10.1186/s43094-020-00095-1
90. Xie BC, Song SY, Xie XY, et al. Isolation, synthesis, and cytotoxicity evaluation of two impurities in nomegestrol acetate. Arch Pharm. 2019; 352(3): 1800295. doi.org/10.1002/ardp.201800295
91. PONDURI R, KUMAR P, VADALI L RAO, et al. Synthesis and Characterization of Potential Phar-

macopeial Impurities of Oseltamivir: An Antiviral Drug. Asian J Chem. 2018; 30(9): 2003-2007. doi:10.14233/ajchem.2018.21376
92. Gebretsadik T, Linert W, Thomas M, et al. LC-NMR for natural products analysis: a journey from an academic curiosity to a robust analytical tool. Sci. 2019; 1(31): 10.3390. doi:10.3390/sci1010031
93. Styles P, Soffe NF, Scott CA, et al. A high-resolution NMR probe in which the coil and preamplifier are cooled with liquid helium. J Magn Reson.(1969). 1984; 60(3): 397-404. doi.org/10.1016/0022-2364(84)90050-7
94. Sturm S, Seger C. Liquid chromatography–nuclear magnetic resonance coupling as alternative to liquid chromatography–mass spectrometry hyphenations: Curious option or powerful and complementary routine tool? J Chromatogr A. 2012; 1259: 50-61. doi.org/10.1016/j.chroma.2012.05.032
95. Tokunaga T, Akagi K, Okamoto M. Sensitivity enhancement by chromatographic peak concentration with ultra-high performance liquid chromatography–nuclear magnetic resonance spectroscopy for minor impurity analysis. J Chromatogr A. 2017; 1508: 163-168. doi.org/10.1016/j.chroma.2017.06.014
96. Huang T, Li H, Zhang W, et al. Advanced approaches and applications of qNMR. Metrologia; 2020; 57(1): 014004. Doi:10.1088/1681-7575/ab336b
97. Singh S, Roy R. The application of absolute quantitative 1H NMR spectroscopy in drug discovery and development. Expert Opin Drug Discovery. 2016; 11(7): 695-706. doi.org/10.1080/17460441.2016.1189899
98. Saito N, Kitamaki Y, Otsuka S, et al. Extended internal standard method for quantitative 1H NMR assisted by chromatography (EIC) for analyte overlapping impurity on 1H NMR spectra. Talanta. 2018; 184: 484-490. doi.org/10.1016/j.talanta.2018.03.003
99. Liu S, Yao S, Zhang H, et al. Determination of relative response factors of cefazolin impurities by quantitative NMR. AAPS PharmSciTech. 2017; 18: 1895-1900. doi.org/10.1208/s12249-016-0654-4
100. Xie B, Liu A, Fang X, et al. Rapid determination of alendronate to quality evaluation of tablets by high resolution 1H NMR spectroscopy. J Pharm Biomed Anal. 2014; 93: 73-76. doi.org/10.1016/j.jpba.2013.07.006
101. Dousheng Z, Yan C, Yaping L, et al. A digitized impurity database analysis method for determining the impurity profiles of gatifloxacin in bulk materials and injections. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2012; 67(10): 827-833. doi.org/10.1691/ph.2012.1162

Hosted file

`Figures.docx` available at https://authorea.com/users/617721/articles/653785-research-progress-on-the-impurities-identification-and-determination-in-pharmaceuticals

Hosted file

`Tables.docx` available at https://authorea.com/users/617721/articles/653785-research-progress-on-the-impurities-identification-and-determination-in-pharmaceuticals