Innovative methodologies for identification and qualification of Impurities: An overview of the latest trends on impurity profiling

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ABSTRACT

Impurity profiling is known to identify, classify and measure both the identified and non-identified contamination present on the medicinal product. Unwanted chemicals which remain or are created during the formulation of medicinal products are pharmaceutical impurities. Impurity profiling helps in the detection, recognition and quantification in bulk products and pharmaceutical formulations of various types of impurities, as well as residual solvents. It is the simplest way to distinguish consistency and stability of bulk drugs and medication formulations. As analytical methodology has developed rapidly, it is essential to consider with their solutions problems related to impurities of drug substances and drug products. Various regulatory agencies including ICH, USFDA, Canadian Drug and Health Agencies stress the criteria for purity and for detecting impurities in active pharmaceutical materials, even in small quantities, as the presence of impurities, may have an effect on pharmaceutical products ' efficacy and health. Therefore, the study focuses on various analytical methods for identification and quantification of impurities in pharmaceutical products to clarify the need for impurity profiling on drug products in pharmaceutical research. To drug regulators, the product substance's impurity profile is a reliable fingerprint to prove that the manufacturing process of bulk drug substances is consistent in quality. The study gives a short summary of recent technical developments in the profiling of pharmaceutical products including pharmaceutical active ingredients as well as pharmaceutical products during 2013-2017. This focuses specifically on a thorough update on various analytical techniques, including hyphenated methods to define and measure thresholds in specific pharmaceutical matrices of impurities and degradants.

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INTRODUCTION

The latest regulations of USFDA, MHRA requires more regulations for profiling impurities than pharmaceuticals. Profiling of impurities by using different methods such as UV, HPLC, LC-MS, GC-MS, and SCFC is carried out. For testing and quantification of impurities, RP-HPLC approach was used. The effect of use of a drug can only be known based on its pharmacokinetic and the toxicological profiles and, therefore, by its adverse effects on mass and dose-like toxicity. Nature of pharmaceuticals must be

observed from the earliest starting point i.e. from crude materials to the finished products.

The present review is an attempt for highlighting some important methods, quality guidelines and applications of impurity profiling. In recent, analyses with high sensitivity and selectivity, and less time for analysis and it is an important part in methodology development. To get these, both methodological and instrumental advantages are required. Many problems have been encountered as regards matrix components and chemical-physical system complexity, as quantitative tests, electronic systems and processing have taken into consideration pesticide or pharmaceutical impurity profiles in order to improve the separation conditions and to achieve precision (precise and correct), sensitivity and selectivity for quantitative analysis; and in this study solvent dilution, buffer pH adjustment, and modifier additions were used to speed up the process for the separations.

According to Webster's lexicon Impurities is something that is unclean or makes something different tainted. A sullied substance might be characterized as a substance of intrigue blended or impregnated with a superfluous or normally mediocre substance (Gorog, 2000).

Various terms have been usually used to portray natural debasements, for example, beginning material, intermediates, penultimate middle (last transitional), by-items, change items, cooperation items related items and corruption items. The United States Pharmacopeia (USP) has diverse areas for debasements incorporating polluting influences in official articles, common contaminations and natural unstable pollutions. These are portrayed as outside substances, harmful contaminations, attending parts, flag pollutions, standard debasements and natural unstable polluting influences (OVIs).

ICH rules classifications impurities such as: natural polluting influences (beginning materials, process related impurities, intermediates and degraded products); inorganic impurities (salts, ligands, catalyst and heavy metals); different materials charcoal and residual solvents (organic and inorganic fluids) . ICH rules on the classification of impurities such as: natural contaminants (beginning materials, process-related impurities, intermediates and degraded products); inorganic impurities (salt, ligands, catalysts and heavy metals); various charcoal and residual solvent materials (organic and inorganic fluids). ICH rules give basic grouping of the impurity influences while none of these can't depict enantiomeric (chiral) Impurity. Chiral impurities have the indistinguishable sub-atomic recipes

and identical availability across various molecules, contrasting only with their particles in space on a three-dimensional game plan. The pharmacological and toxicological profile distinctions have been seen with chiral impurity in vivo (Daniels *et al.*, 1997).

In this manner, it is very clear that the items planned for human utilization must be portrayed as totally as could reasonably be expected. Observing and controlling of impurities for the most part gives affirmation of the quality and wellbeing of a drug. In this way, the explanatory exercises concerning impurities, in drugs are among the most critical issues in current pharmaceutical investigation. Expository checking of impurities in new drug substances is a key part of the current rule issued by the International Conference on Harmonization (ICH).

Albeit distinctive books and survey articles have been distributed to outline the investigation on impurity, yet there is no give an account of late years which empower to portray the current expository viewpoints of pollution and corruption profiling. On keeping this view in the psyche, show work has been meant to audit the scientific patterns for constrained contamination profiling of dynamic pharmaceutical fixings and pharmaceutical medication item (Ahuja and Alsante, 2004; Smith and Webb, 2007).

Articles distributed on polluting influence profiling amid , were broadly looked into and changed parameters, for example, grid of examination, helpful classification of the medication, introduce debasement section determination utilized for partition, portable stage piece utilized for elution, mode and additionally wavelength of location and year of production were accounted to set the current pattern in investigative point of view (Baertschi, 2006).

Trends in 2013

Profiling of impurities by SFC and RPLC

In this review article both double and triple combination of antiretroviral drugs were used for the isolation of impurities by using both SFC and RPLC techniques (Lamivudine, BMS 986001, Efavirenz). Here the stationary phase was Princeton 2-ethyl pyridine and mobile phase used in the combination of 10mM ammonium acetate and methanol with 0.1% isopropyl amine. Further useful to decrease the peak tailing. For the RPLC isolation was achieved by using stationary phase. Discovery HSF5 and the mobile phase as 10mM ammonium acetate, pH 5.5 and methanol. In this procedure gradient elution technique was used. Some advantages and disadvantages are obtained in this technique. In this technique 3 active pharmaceutical ingredients and around 13 possible impurities were selected and all
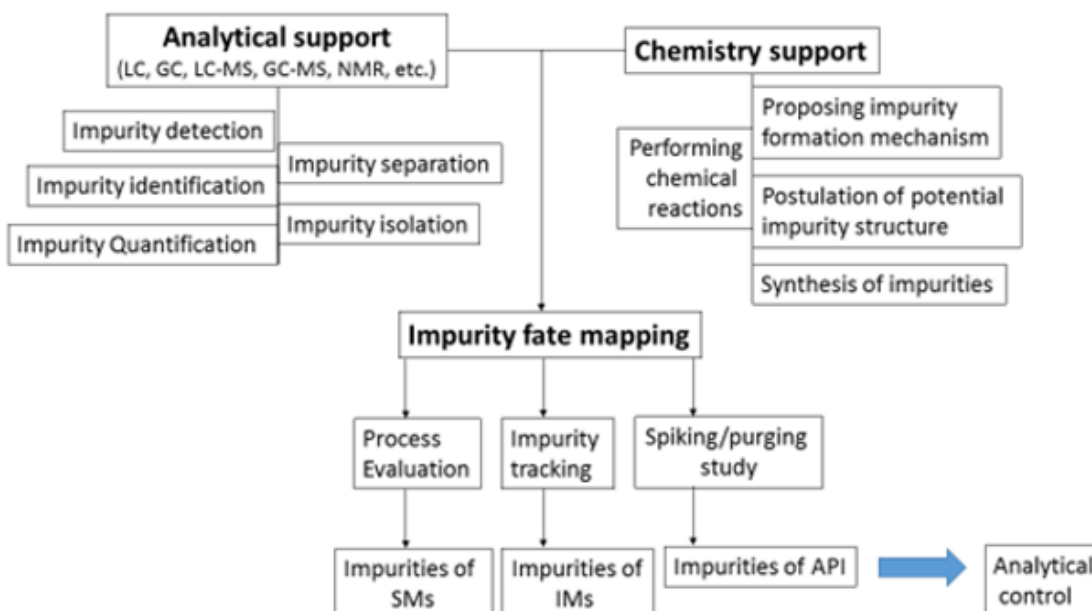
Figure 1: The flow of identification method of the impurity analysis.

these are resolved by SFC & RPLC and 15 peaks were eluted and orthogonality was achieved relative to RPLC. The results which got were got are satisfied and obtained results are validated (Alexander *et al.*, 2013).

Profiling of impurities by HPLC and MS with an electrospray ionization (ESI)

A robust HPLC method was developed for olmesartan medoxomil in both pure form and in marketed formulation also. Here they used kromosil C18 columns, disodium hydrogen phosphate: acetonitrile (60:40 v/v) as a mobile phase. The detection was performed at 225 nm. They were linearity around 2-7μg/ml the accuracy for olmesartan was 100.73%. The relative standard deviation (RSD) of the Inter and intraday assay was less than 0.71 per cent in drug form and 1.10 per cent in tablet dosage form for impurity of Olmesartan (Pai and Sawant, 2013).

Profiling of impurities by RPLC and MS

In this research a impurity profiling method were developed for micronomicin sulphate by using RPLC and MS method. They used an C18 column which is of reversed phase and then they were coupled it to an mass spectroscopy equipped with an electrospray ionization (ESI) source in the positive ionzation mode which provides MSn capability, 36 samples were analysed and 5 impurities were detected in accordance with references, 11 of which were identified in reference with relevant literature, 20 new impurities were identified using MS spectra's and then they were compared with standard ones. This strategy was applied to evaluate the quality parameters for the micronomicin sulphate injection from other different manufacturers (Yuan *et al.*, 2013).

Profiling of impurities by UHPLC and HRMS

For profiling of manufactured thyroid hormones and its impurities, an ultrahigh-performance fluid chromatography (UHPLC) hyphenated with high-determination mass spectrometry (HRMS) was used. Five distinctive bunches of an engineered thyroxin were investigated bringing about the identification of 71 impurities inside 3min add up to examination time. By precise mass estimates, PC-based atomic formula calculations, multi-stage highdetermination mass spectrometry (HRMSn) and nuclear magnetic resonance spectroscopy enabled the distinguishing evidence of 71 impurities, 47 of which were previously obscure. Thirty of the last were essentially demonstrated as new class of thyroid hormone subordinates, including tests of deionisation, aliphatic chain oxidation, and dimeric mixtures. For the negative electrospray ionization mass spectrometric recognition in complete sweep mode, position confinement points for the thyroid mixes were in the 6 ng / mL range. Within daytime and daily rehash capacities of maintenance times and pinnacle zones were below 0.5 per cent and R.SD 3.5 per cent. The performance attributes of the technique to the extent that vigour and the information content clearly show that UHPLC-HRMS is satisfactory for rapid and solid discovery, distinguishing evidence and semi-quantitative assurance of the following impurity levels in manufactured pharmaceuticals (Neu *et al.*, 2013).

Profiling of impurities by GC/FIC and GC/MS

An impurity profiling method was developed in another research paper, and they found that some methamphetamine (MA) crystals contain pharmaceutical impurities. This is due to ephedrine and pseudoephedrine these impurities were difficult to encounter. These impurities were identified by GC/FIC and they were identified by GC/MS method. These can be co-ingredients of the legal drugs used as an ephedrine or pseudoephedrine source.

Conversely, some are presumed to be added during or after clandestine synthesis as adulterants. It is interesting to note that some of these have been found in other countries in the MA crystals which were confiscated the same year. Especially in 2010 species of pharmaceutical impurities increased in MA crystals, indicating a change in precursor chemicals and/or sources of production (Choe *et al.*, 2013).

Profiling of impurities by RP-HPLC ,1H NMR

Simple and precise stability indicating the dronedarone analytical strategy had been developed and approved using RP-HPLC process. The developed technique was used to determine the dronedarone medicate substance and tablets (Multaq ®) measurement and related substances. According to the International Conference on Harmonization (ICH), the medicinal substance was subjected to stress conditions such as hydrolysis (acid and base), oxidation, photolysis and warm degradation to demonstrate the stability-indicating the concept of the technique. Essential degradation was observed with hydrolysis of the acid and base, and degradation of peroxide. Between acid and base hydrolysis, and degradation of peroxides, the Biggest Critical debasement was seen. The key degradants were characterized by LC-MS, FTIR and 1H NMR spectral tests. LC-MS, FTIR and 1H NMR spectral analysis identified the main degradants. The chromatographic conditions have been advanced by using an unclean arrangement and examining examples from confined corruption. Determination between dronedar-one, process-related contamination (in particular Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp-6, Imp-7, Imp-8, Imp-9, Imp-10 and Imp-11) and degradation products was observed to be more prominent than 1.5 in the HPLC technique developed. The on-line column of Ascentis Express C18 (4.6 = 10 cm i.e., particle size 2.7 μm) with flow rate 1.2 mL·min–isolates the eleven potential process associated polluting influences. The LC method used a linear elution of gradients and a wavelength of detection of 220 nm. Various solvents, temperatures and pH levels were analysed for chromatographic behaviour of the considerable amount of contamination (Landge *et al.*, 2013).

Profiling of impurities by SUNFIRE C18 column

For the simultaneous estimation of impurities of Guaifenesin and Dextromethorphan in pharmaceutical formulations, a sensitive, stable gradient RP-HPLC method had been developed. Effective chromatographic separation were accomplished on a Sunfire C18, 250 × 4.6 mm, 5 μm column with mobile phase involving a gradient mixture of solvents A and B. The mobile phase flow rate of 0.8 mL min−1, and the wavelength was 224 nm. The regression analysis shows that for guaifenesin, dextromethorphan and their impurities the values r (correlation coefficient) were more noteworthy than 0.999. The oxidative, corrosive, base, hydrolytic, thermal, and photolytic stress conditions were evaluated in both guaifenesin and dextromethorphan. Guaifenesin was found to be stable and dextromethorphan was found to fully degrade under peroxide stress. Guaifenesin, Dextromethorphan and their impurity have all settled down the degradation products. The peak purity test has been verified by the homogeneous and irregular Guaifenesin and Dextromethorphan crest in every stress sample, and the mass adjustment indicates a force exhibiting the strength of the strategy at 98 percent. ICH rules on specificity, linearity, LOD (Limit of Detection), quantification, robustness, precision, and basic approval were given for the developed technique (Raju *et al.*, 2013).

Trends in 2014

Profiling of impurities by UV and GC-MS techniques and a RP-HPLC methods

The current research work was coordinated to characterize and evaluate regionic nimodipine impurity, i.e. diethyl 1, 4-dihydro-2,6-dimethyl pyridine dicarboxylate, UV, IR, NMR, and GC-MS techniques as well as an ICH Q2B method, to measure the amounts of 1, 4-dihydro-2, 6-dimethyl-4-(p-nitrophenyl) pyrrrhyl (pyrhythene) in accordance with the guidelines laid down in ICH Q2B.

Methods: NI's synthesis was made through the use of p-nitrobenzaldehyde, ethylacetoacetate and the proximity of alkali and methanol as a catalyst for Hantzch pyridine synthesis. The rate of yield was 89.29 percent. NI filtration and recrystallization have been done. The preparatory evaluation was conducted by melting points, elemental analysis and TLC on the scale of the research centre.

Results: 156-158 C was measured as a melting point of impurity. By the use of Chloroform: methanol

(9:1), and the Rf was shown to be 0.79, transmitted TLCs of impurity. In addition, modern methods like the FT-IR, NMR (13C and 1H), the GC-MS were applied to assert the structure of the NI. The NI in NiDipine bulk and formulation in line with ICH Q2B guidelines was developed with a RP-HPLC method. ICH guidelines was used to verify the process.

Profiling of impurities by 1H and 13C NMR

Reverse Phase chromatographic electrospray mass spectrometric methods for process related impurities and forced Efavirenz degradants of bulk medicines have been developed and validated. Degradation experiments have been conducted on Efavirenz. In alkaline hydrolysis a mass-based purification system was used to identify enormous degradation, described in high-determination mass spectrometry, positive electrospray ionization couple mass spectrometry and 1H and 13C NMR spectroscopy. Exact mass estimation and NMR spectroscopy revealed the conceivable structure of process-related and pressurized impurities. Waters bondapak C18 column (250 mm — da 4.6 mm, 5 m) refined the satisfactory spacing, with a flow rate of 1.0 mL/min of 5mM ammonium acetonitril as a mobile phase. Effavirenz, degradants, and process-related impurities had Eluents detected by diode array detector at 247 nm, and measurement limits of 0.1 –2.5g/mL. The process for liquid chromatography was approved under the International Harmonization Conference Guidelines (Gadapayale *et al.*, 2015).

Profiling of impurities by counter-current chromatography (CCC) and preparative HPLC

Impurity profiling was done alongside the drug identification process. The advent in development of honokiol and quercetin as anticancer medication hopefuls, a reliable counter-current chromatography (CCC) and preparative HPLC were utilized for the contamination profiling and recognizable proof of honokiol and quercetin. Some execution parameters, such as column length, sample loading limit, separating time, solvent consumption and test efficiency, have been analyzed, taking into account the final objective of evaluating the efficacy of separation.

They found that the example stacking limit and along the limits of preparative HPLC were not tasteful, while CCC given bigger example stacking (particularly for an example with poor dissolvability), expended less dissolvable and created higher output than preparative HPLC. By this method about Six impurities of the compound honokiol including one new compound were separated (Li *et al.*, 2014).

Profiling of impurities by HPLC-CAD

The Mucolytic and anti-inflammatory drug carbocisteine was subjected for impurity profiling by using HPLC-CAD, charged aerosol detection being its detector. This method was developed and validated according to the ICH guidelines Q2 (R1). The linearity were generated having the R^2 0.995. Concentration range being (0.05-0.25 ug). The detection limit of 0.03% were recorded. Separation were done using C18 column, mobile phase being trifluoroacetic acid 10Mm and acetonitrile 12% (V / V). Volatile impurities such as choloroacetic acid and unstable cysteine could be only determined quantitatively by NMR instrument, maleic acid being the internal standard and UV spectroscopy after reaction with Ellman's reagent respectively. Six batches were tested from three manufacturers. The purity varied between (99.0 and 99.8%). The principal impurités are cysteine and N, and the by-product of synthesis is S-dicarboxymethylcysteine (Wahl and Holzgrabe, 2014) .

Trends in 2015

Profiling of impurities by SFC

Supercritical Fluid Chromatography is a technique for separating chiral drugs on both an analytic and a preparatory scale, which is already well known in the pharmaceutical industry. For drug impurity profiling by the use of SFC is explained here. The starting point in drug impurity profiling method development is selection of dissimilar stationary phases. There, the retention factors (k39) were used for 64 drugs in a set of different columns, and they measured it by 27 columns in SFC. Here they maintained a 150 bar pressure at 25°C and CO2 and methanol with 0.1% sopropylamine (5-40% over 10 minutes) as a mobile phase at a flow rate of 3ml/min. On the one hand they used main component analysis and on the other hand they used k-values to calculate the correlation coefficients. They were used colour maps were used to select a set of 6 dissimilar stationary phases. The results got from stationary phases were matched with literature articles. Compound retention mechanisms were assessed and a mixture of compounds with similar properties and structures were evaluated for 6 stationary phases.

Profiling of impurities by LC/MS

The chromatographic Purity Profiling (CPPs) is the name for the detection, identification and determination of substances and other impurities in active pharmaceutical and finished dosage forms (FDFs) have applications in analytical and chemical metric applications. CPP is used to print and separate samples from each other. The market for morphine and

other types depends more and more on the demand for natural opiates. This study were carried out to identify sources of morphine. Here 28 samples were examined by using LC/MS method. Different recognition techniques were used for the classification and authentication purposes. Here they used the chromatographic purity profile combined with the multivariate data analysis for fingerprint analysis of morphine samples (Acevska *et al.*, 2015).

Profiling of impurities by GC-MS

Objective: API S and processing by-products in methamphetamine samples have been identified in this report. As methamphetamine and the misuse of its impurities are not unnoticed in the body, the other purpose of this research was to address health effects associated with impure methamphetamine abuse in a short review.

Here 53 samples of methamphetamine were carried out by using gas chromatography/mass spectrometry method. The reports of methamphetamines include methamphetamines, amphetamines, ecstasies, phenmetrazines, pseudoefedrines, tramadols, acesic acids, benzaldehydes and other chemicals. The methamphetamine crystals contain various chemical impurities from processing and have been added with API. Besides the environmental threat posed to people who are in direct and indirect contact with these pollutants in methamphetamine laboratories (Etemadi, 2015).

Profiling of impurities by GC/MS

In this analysis, a profiling of impurity for spilled benzene compounds was performed. In this analysis, toluene was used. The GC / MS method was employed here to classify and measured impurities. In this method 8 samples were collected from different manufacturers and they were analysed by using GC/MS method. This approach has then been developed combined with dispersion, seamlessness analysis and t-test. The results showed that the delivery system of the original toluene samples may be separated from various manufacturers. The weathered toluene samples can be effectively identified through the similarity estimation and t-test methods (Han *et al.*, 2015).

Profiling of impurities by Flame spectrometric method

These are antimalarial formulations of Artemether-lumefantrin (AL) that had been determined and 6 samples from pharmacy were bought and tested by the flame spectrometry method using the Elementary impurities of Paediatric Supply Powder (PPS) and Double-Strength Tablet (DST).The levels of heavy metals were compared with standard limits. This comprises about 10 metals and amounts of PPS and DST in both paediatric and adult formulaments, ranging from 0.001-0.016 and 0.001-0.017ppm. Of the 6 products one was significantly higher, relative to the respective forms of formulation (p<0.05), with cadmium, copper, chromium, nickel and cobalt. The levels of compounds namely chromium, nickel, and cobalt were significantly affected but can't be distinction was made when DST and PPS products was compared with the levels of cadmium, plum, zinc and arsenic (p<0.05). Elemental impurities will present in less amount in the oral suspension than the standard values and they are in a safe range to use (Awofisayo *et al.*, 2015).

Trends in 2016

Profiling of impurities by GC-MS

The simple and reliable method for identifying 3, 4-MDMA impurities in ecstasy tablets by gas chromatograph mass spectrometry (GC–MS) has been developed. Eight impurity samples were obtained in alkaline diethyl ether and then analyzed with GC–MS. The results showed a high level of MDMA of 37.6% to 57.7%. A strong recognition of 3-mDMA,4-methylenedioxyamphetamine(MDA), amphetamines (AM), methamphets (MA), and ketamin (Keta), respectively was shown by the GC-MS technique. The GC-MS method showed that the trial results showed the time window that was appropriate without interfering with peaks. GC-MS has been found to be an effective and rapid (Jalali *et al.*, 2016).

Profiling of impurities by HPLC and 2D NMR

Developed a method by HPLC for the determination of Vildagliptin, a member of a new class of oral anti-diabetic drug. One unknown impurity was also identified in the range of 0.01–0.06% in different laboratory batches of vildagliptin along with known impurities. The structure of unknown impurity was proposed using LC / ESI –MSn as (2S)-1-[2-[3-hydroxyadamantan-1-yl) imino] pyrrolidine-2-carbonitrile (Impurity-E). The unknown impurity were found to be unstable in diluent (H2O: CH3CN) and degrading into another stable impurity. A preparative liquid chromatography isolated the degraded stable impurity from the enriched reaction sample. FT-IR, NMR (1H, 13C and DEPT), 2D NMR (HSQC, HMBC and COSY) and mass spectral data (8aS)-3-hydroxy-octahydropyrrolo[1,2-a]piperazine-1,4-dione (Impurity-F) were used to determine the structure of stable impurity. Impurity detection, unusual impurity-E behaviour, impurity-F isolation, fragmentation mechanism and structural elucidation were also addressed (Kumar *et al.*, 2016).

Profiling of impurities by HPLC

Developed and validated Method for Methionine determination by HPLC. It is synthetically generated. Other than oxidation and dimerization products, impurities were also by-products of synthesis. Method tested using column SIELC ® Primesep 100. Impurities were isolated by reversed phase with cationic mechanism of exchange on the mixed mode column. The detection limit was 0.06–0.30 μg / ml (0.0004–0.002 %), the quantification limit was 0.30–0.75 μg / ml (0.002–0.005 %) and the linearity was 0.3–30.0 μg / ml (0.002–0.200 %). The approach was found to be effective (intermediate RS < 5 %; n= 2) and accurate (96.0–121.4 % recovery; n = 3). The process is also ideal for the DL-methionine and D-methionine purity measurement. There were very low levels of impurities in samples. It could only be observed with less than 0.05% for L-methionine-sulfoxide and N-acetyl-dl-methionine (Kühnreich and Holzgrabe, 2016).

Profiling of impurities by TLC-densitometry and HPLC

The compounds Ibuprofen (ASP) and dipyridamole (DIP) as a mixture in pharmaceutical formulations used for treatment of strokes. In this combination tartaric acid was used as an excipient (in DIP pellets plan for supported discharge), which builds the likelihood of arrangement of dipyridamole tartaric corrosive ester contamination (DIP-I). Here salicylic acid (SAL) was found to be the synthesis impurity and a degradation product of ASP. In this work, two chromatographic methods were used namely, TLC-densitometry and HPLC, which have been built up and validated for concurrent assurance of ASP, DIP, SAL and DIP-I. Great detachment were accomplished by utilizing silica gel-g as stationary stage and toluene: methanol:ethyl acetic acid derivation (2:3:5, by volume) as portable stage on account of TLC-densitometry and Zorbax ODS section with versatile stage comprising of phosphate cushion (pH 3.3): acetonitrile: triethylamine (40:60:0.03, by volume) for HPLC. Impact of various natural solvents in portable stage structure had been concentrated to upgrade the partition effectiveness in TLC densitometry. Components influencing the proficiency of HPLC, similar to pH of the cushion were utilized, natural dissolvable proportion in the versatile stage and stream rate, had been painstakingly examined utilizing one variable at any given moment approach. At last, the proposed techniques were validated according to ICH guidelines (El-Ragehy et al., 2016).

Profiling of impurities by UHPSFC and UHPLC

The newest antidepressant is agomelatine. This provides a whole new approach to treating depressive disorders. Two chromatographic methods have been developed for agomelatin determination and its impurities. The separation was done by using stationary phase based on BEH 2-EP and gradient elution with CO2 and methanol containing 20 mM ammonium formate and 5% of water. The UHPLC separations were performed on stationary phase BEH Shield RP18. The mobile phase used was a mixture of acetonitrile and methanol in ratio 1:1 and ammonium acetate buffer pH 9.5.

Both methods developed were properly validated according to the ICH Guidelines The UHPSFC method were linear in the range 0.25–70 μg / ml for all analyses with method accuracy $\geq$97.4% and $\geq$100.2% and method intra-day precision RSD $\leq$2.4 and $\leq$0.8 for impurities and API (active pharmaceutical ingredient), respectively. The UHPLC method was linear in the range 0.1–10 μg / ml for all analytes except three impurities for which the linear range was larger 0.1–25 μg / ml. Method accuracy $\geq$95.7% and $\geq$95.2% and method intra-day precision RSD $\leq$2.6 and $\leq$1.5 were found for impurities and API, respectively.

Tablet sample measurements have been carried out and the selected method parameters have been compared. In sum, the methods and its impurities in pharmaceutical quality control (QC) were sufficient in determining agomelatin while, in particular, the UHPSFC approach was found to be more convenient during the method development phase. The advantages of newly developed UHPSFC-PDA (photo diode array detector) method was its environmental friendly because of the mobile phase used, which had a excellent resolution, selectivity and high speed of analysis (total time of separation were 4.1 min) (Plachká et al., 2016).

Profiling of impurities by LC-MS

A RP-LC column (50 mm long, 2.1 mm i.d., 1.9 μm) was used to characterize unknown degradation products from mycophenolate mofetil produced under oxidative stress conditions by LC-MSn. The mobile phase consisted of 26% acetonitrile and 74% 9 mm ammonium acetate pH 5.87, the temperature at the column was about 40 ° C and the mobile phase flow rate was 400 μL min-1. Four products of degradation formed after an hour under 15% of hydrogen peroxide. In the past, two mycophenolic acid degradation products (m / z 321) and mycophenolate n-oxide mofetil (m / z450) were known In order to identify their structures they have been thoroughly examined by both other unknown degradation materials, DP I (m / z 319) and DP II (m / z466). The fragmentation patterns of the

mycofenolate mofetil and the N-oxide of mycofenolate mofetil have been established, analyzed and compared, and thus the structure of the unknown product for the degradation has been elucidated. The oxidized unsaturated mycophenolate aldehyde was proposed as the unknown degradation product DP I (m / z 319.2), which was also an ammonium adduct ion in the range m / z336.3.The second unknown degradation product, DP II (*m/z* 466.3), were characterized as a doubly oxidized derivative of mycophenolate mofetil. A possible degradation pathway of mycophenolate mofetil under the influence of an oxidizing agent were also proposed (Golubović *et al.*, 2014).

Profiling of impurities by HPLC and mass spectroscopy

One of the recent developments in the area of pharmaceutical formulation is the topical formulation alliance of cyclobenzaprine, hydrochloride, piroxicam and lidocain. A chromatographic reversephase method for the impurity of cyclobenzaprine chloride, lidocaine and piroxicame in the topical semisolid formulation has been developed in this report. In addition to 2,6-dimethylamine,2-pyrydilamine, cyclobenzaprine hydrochloride impurities (dibenzosuberenon, amitriptyline, carbinole, cyclobenzaprine N-oxide and anthrachinone) were determined. Impurities were also reported. The target compounds were characterized using an acetonitrile:methanol (60:13:27, v / v / v) in a mobile phase consisting of a phosphate buffer mixture (0.025 M; pH 6.2). About three additional degradation products have been identified. Mass spectrometry identifies the unspecified impurities and allows the association with the potential source compounds to be used with the correlation matrices. In order to confirm precision, linearity, consistency, and accuracy, the chromatographic conditions were certified and validated according to ICH guidelines (Cioroiu *et al.*, 2016).

Profiling of impurities by GC/MS

A Simple gas chromatography–mass spectrometry (GC / MS) technique used for determining the antihistamine medication dimen hydrinate (DMH) was developed in the presence of six of its associated substances and possible impurities, namely diphenyl methane, diphenyl methanol, benzophenone, orphenadrine, caffeine, and8-chlorocaffeine. In electro-impact (EI) mode, the method was used for resolution of the subividated compounds using a trifluoropropyl methyl-polysiloxane (Rtx-200) capillary column. In less than 15 minutes, excellent baseline isolation of DMH and the associated substances cited was achieved. The parent DMH drug

quantification was based on its peak range estimation. The method was evaluated in terms of the linearity, the range, accuracy, precision, specificity, robustness, detection and measurement limit for its reliability and analytical efficiency. The DMH calibration curve was linear with a measurement coefficient (R2) of between 50-500 μg / mL= 0.9982. For the DMH test in tablets with recoveries > 96.80%, the proposed approach has been used successfully (Belal *et al.*, 2016).

Trends in 2017

Profiling of impurities by supercritical chromatographic method

A supercritical chromatographical approach was developed and optimized for the separation of a drug and its impurities, using the experimental design process and simulation of chromatograms. Optimisation of the additive and injective solvent composition was accompanied by stationary phase screening. A DoE technique has been used to simultaneously regulate the column temperature, backpressure and the gradient slope. Regression models have been constructed for the retention times and the peak widths of each portion. Factor rates were then used for different grid points to predict mixture components retention times and maximum widths using regression models and the best separation was calculated for the worst separated peak pair in the experimental area. A plot of minimal resolutions was used to evaluate the factor rates between adjacent peaks, which led to the greatest resolution. The results of the DoE variables were visualized so that the analytical chemist knows how to model the resulting chromatogram. In less than eight minutes, the 3 compounds of a single active ingredient and 7 impurities were isolated. The methodology discussed in this paper shows how SFC approaches can be effectively established and implemented using simple concepts and tools. 46] This study analysed the methyl one prepared from catechol synthesis and organic impurity profile.. The primary objective of this research was to evaluate the synthesis of 3,4-methylenedioxypropiophenone using the synthesized methyl one analysis. The second goal was to detect organic impurities in methylon and secondary compounds in the structural elucidation and origin. GC-MS and NMR spectroscopy were used to detect organic impurities in the reaction materials. Six organic impurities were found in 1,3 benzodioxols and identified as 1,3 benzodioxole dimer,[1, 3] dioxol[4,5-b] oxanthren, 4,4,5'-, 5,5'-methylenebis-1,3-benzodioxolen. In 3,4-methylenedioxypropiophenone, 6

organic impurities were found and identified in the form of (2-hydroxyphenyl)propanoates (2-(chloromethoxy)-phenyl)propanoate, (3-propanoxypropano-5-yl)propane(2-benzodioxy-3-1-enyl)prophanyl), (6-(1,3-benzodioxol-6-methyl-cyclopenta[f][11,3]benzodioxole). Exploratory synthetic studies have found organic impurities in 3,4-methylenedioxypropiophenone unambiguously. In 5-bromo-3,4-methylenedioxypropiophenone two organic impurities have been identified,[2-(chloromethoxy)phenyl] and 3,4-methylenedioxypropiophenone. In methylone 5 organic impurities had been identified, which include, 3:4-methyleneedioxypropiophenone, 1-(1,3-benzodioxol-5-yl), 1-(1,3-benzodioxol-5-yl)-N-methyl-1-imine, 1-(1,3-benzodioxol-5,2-diimin, and butylated hydroxytoluene. The origin of these organic impurities was also ascertained, providing valuable insight into the chemical profiles of methylone and the intermediate compounds. Nevertheless, the organic impurities contained in the synthesized methylon using standard techniques could not be used to classify the catechol or the intermediate 1,3-benzodioxole.. This shows that organic impurity profiling for methylone has limitations in evaluating the natural and synthetic pathways used for precursor determination (Heather *et al.*, 2017).

Profiling of impurities by RP-HPLC and high resolution tandem mass spectrometry (HRMS/MS)

An comprehensive study was conducted for the first time using reversed HPLC process and advanced structural elucide techniques including high Resolution Tandem-Mass (HRMS / MS) and online hydrogen-deuterium (H / D) exchanges to investigate the impurity profile of synthetic Thyroid API (active pharmaceutical component) liothyronine sodium (LT3Na). LT3Na In total, there were 39 characteristic compounds, of which 25 were previously unaccounted for in the literature. In the newly characterized sodium impurity of liothyronin, a wholist thyroid API impurity classification system (WAPI) may be applied in the newly developed closely associated API levothyroxin sodium (LT4Na). Additionally, detailed fragmentation mechanisms addressed and rationalized the mass-spectrometric CID fragmentation of different associated substances. In addition, to corroborate assignments of chemical structures derived from MS results, UV / Vis absorption characteristics of the API and selected impurities have been studied (Ruggenthaler *et al.*, 2017).

Profiling of impurities by Supercritical fluid chromatography

The supercritical fluid chromatography in the field of fluid separation sciences has increasingly been recognized as a single and efficiency technique. The importance of SFC on the quality control of pharmaceutical products in particular with regard to the determination of the active pharmaceutical ingredient (API) was demonstrated in recent studies. Furthermore, quality control often needs impurities to be calculated. The aims of the present work were: I to demonstrate the importance of SFC in determining impurities in salbutamol sulpfate API as a reference technique; (ii) to suggest an alternative to a European pharmacopeium reference HPLC process (EP) involving ion-pairing reagent. Firstly, the most suitable and selective stationary process has been screened.. Second, the approach has been developed using design space methodology in the context of a robust optimization strategy. Salbutamol sulphates and associated impurities were separated in 7 minutes, seven times faster than the European Pharmacopeia LC-UV method proposed (total running time of 50 min). Ultimately, the complete validation of impurities B, D, F and G in raw material salbutamol sulfate has been successfully achieved using an accuracy profile method. The dosage range validated was 50 to 150% (corresponding to 0.3% concentration level), and LODs were measured as close to 0.5 g/ml. In order to quantify salbutamol impurities, the SFC approach proposed in this study could be provided as an appropriate rapid alternative to the EP LC method (Dispas *et al.*, 2017).

Profiling of impurities HPLC and ESI–MS, 1D NMR

About eight impurities (CLB Imp-A to CLB Imp-H) were found as a result of the optimization of the process using a gradient HPLC system with UV detection in just a few of the laboratory batches used as an anxiolytic agents. According to the co-spiking study, however, six impurities (CLB Imp-A to-F) identified in European Pharmacopeia were harmonized and found to be two impurities completely unknown (CLB Imp-G and -H) which were not mentioned in the previous literature. These two new impurities structures have been alleged to be 8-chloro-1-methyl-5-phenyl-1,5-dihydro-3H-1,5-benzodiazepine-2,4-dione (CLB Imp-G) and 5-chloro-1-methyl-3-fenzo-1H-benzo[d]imidazol-2(3H)-one (CLB Imp-H) based on the LC-ESI / MSn trials. Synthesis of the alleged impurity structures was confirmed following full spectral analyzes including ESI–MS, 1D NMR (1H, 13C and DEPT), 2D NMR (HSQC, HMBC and COSY), and chromator retention time. Identification, synthesis, structural features, the production and control prospects of these new impurities have been described in detail and stated first in this document (Kumar *et al.*,

Profiling of impurities by IR and NMR spectroscopy

Unknown impurities associated with the dapoxetine base degradation cycle were isolated. The structural description of this compound is provided using precise mass data, IR and NMR spectroscopy. Clearing of dapoxetine-N oxide, the main oxidative and metabolic degradation agent of dapoxetone, resulted in unambiguous resonance assignments to cinnamyloxynaphthalenes forming geometric isomers. In order to obtain the proposed degradation pathway and structural structure of the degradation products, a reliable and simple synthetic approach was developed first time for the synthesis of dapoxetine N-oxide and cinnamyloxynaphtalene. It was observed that (2E)cinnamyloxynaphthalene is the main degradation agent for an airexposed dapoxetine base, while its Z isomer has also been shown to be of minor impurity (Darcsi *et al.*, 2017).

Profiling of impurities by coupling two achiral stationary phases

In order to obtain the fullest impurity profiling of synthetic medicinal products with a single chromatographic technique, a high resolution is required that combines a high efficiency and flexible selectivity, thus enabling the most similar analytics to be segregated. While comparison to a single column chromatographic process, the coupling of the complementary stationary phase promises to improve both efficiency and selectivity. The use of long columns is enabled by the low viscosity of the mobile process with supercritical fluid chromatography (SFC). We examine the importance of coupling two stationary achiral (Acquity UPC2 HSS C18 SB and Nucleoshell HILIC) phases that have been observed previously in SFC for carrying out profiling impurities on 25 different pharmaceutical substances with different numbers and amounts of impurities. The single-column gradient methods are compared to two alternative column gradient methods (phase C18 in first or second position) based on selectivity, peak efficiency, susceptibility, UV-estimating pharmaceutical pureté of the active ingredient and amount of UV-estimated impurities > 0.04 percent. The combination of two columns in a single study appears to be more advantageous than two consecutive methods in the single columns. The total time of analysis is almost same, but in about 35% cases with more detailed chromatograms (West *et al.*, 2018).

RESULTS AND DISCUSSION

Many methods have been developed for the separation and identification of the impurities present on the pharmaceutical dosage forms. The separation is based on the drug and the nature of the impurity present. More over based on the solubility, polarity, pka characters are considered and acidic, basic and neutral conditions are provided to facilitate the separation on the stationary phase. The isolation of the impurities can be done through solid phase extraction (SPE), supercritical fluid extraction (SFC), capillary electrophoresis (CE),

Super critical chromatography (SFC). Further the identification can be performed by UV, IR, MS, NMR spectrometry techniques. The advent of hyphenation these like LC-MS, LC- NMR, LC-MS/MS, GC-IR, GC-MS provide more versatility, sensitivity and provide rapid estimation of the impurities present in the API. The Figure 1 depicts the flow of identification method of impurities present.

Out of which HPLC has the key role. Where a clear idea about the mass balance measurements is necessary to consider the degradation pathways and product structures. When using HPLC, there are about seven theoretical sources of "mass imbalance," found and there are practical ways for resolving these problems.

To establish responsible mass quantitative potential cause of mass imbalance, an idea of both reactant and product structure nature is crucial (Alsante *et al.*, 2014).

1. If the Impurities do not elude from column HPLC

Using HPLC gradient for wide range of polarities and longer holding time with the best mobile phase conditions.

Reverse phase analysis of samples.

Estimation of the sample separation through hydrophilic interaction liquid chromatography (HILIC) and by using electrophoresis techniques.

2. If the Impurities are poorly separated and are "missed"

By using HPLC with technique of steep gradient.

Use of HPLC with isocratic separation with a high concentration mobile phase conditions.

Using of UV spectrophotometry without experimental separation technique.

3. Where the impurities co-elute with the parent compound

Change column, solvent, or gradient phases of HPLC.

Use an orthogonal (different) separation method.

Look for peak purity using a UV -PDA detector and LC/MS techniques.

4. If the Impurities can't be detected by the detector

Using a UV-PDA (200–400nm) detection to monitor at low to high wavelength ranges.

By using of UV-transparent clear solvents and buffers solutions.

use various alternative detector e.g., evaporative light scattering detector (ELSD), chemilumines-cence nitrogen detector (CLND), corona, charged aerosol detector (CAD).

Analyzing the sample by using alternate or orthogo-nal detection method.

Use a reverse phase (RP) or a normal-phase (NP) thin-layer chromatography technique (TLC) – Use various options / chemistries for developing TLC plates or fluorescent-impregnated TLC plates.

5. There is poor analytical recovery of the impurities

Consider insolubility of impurities in analytical phases.

Proper visual identification and observation.

6. In case of improper analytical recovery of the parent molecule

Prepare different solvents in various proportions for the sample preparation.

Separate and filter the solid residue and analyse using other analytical technique (e.g., probe-MS)

Predict the potential for insoluble excipient reac-tions.

Check the volatility of the solvents used.

Change to other analytical techniques which can provide the required results (e.g., GC-headspace).

Predict the adsorption losses while analysing the sample solution(e.g., photosensitivity).

Perform the method on different container and vali-date the results. (e.g., glass and polypropylene mate-rial).

Improve the extraction efficiency of solvent used by altering the pH for elution of the compounds.

Identify the potential cause of the instability in the prepared standard and test solutions.

7.If the variations in response variables contribute to unreliable quantification.

Review the detected impurities from the UV spec-trum.

Consider alternate detectors-MS, Corona CAD, CLND, FID, LC / NMR, the evaporative light scatter-ing.

Determine response factors.

Isolation, purification, and resolve by new tech-niques.

Use CLND or CAD techniques (without isolation of impurities).

Intend the use of Magnetic nuclear resonance quan-titative (qNMR).

CONCLUSIONS

The present survey depicts far reaching refresh on late patterns in investigative points of view of degra-dation and impurity profiling of pharmaceuticals amid 2013-2017, which may serve and test view to the entire intrigue gathering. The main aspect for the profiling of the impurities is to detect the new entity over its threshold limit of 0.1% in the pharma-ceutical formulations thus the analyst performing the quantification have to utilize the correct meth-ods which have high selectivity nature. An accurate method allows proper identification of the impurity present, the establishment of the guidelines allows the manufacture to provide the quality aspect of the API.

Conflict of Interest

None.

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