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A REVIEW ON CAPILLARY ELECTROPHORESIS METHODS FOR IMPURITY PROFILING OF DRUGS

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ABSTRACT

Capillary electrophoresis is extensively worn for the impurity profiling of pharmaceutical compounds that mainly accommodate stereochemical centers in their structure, analysis of biomolecules and physical properties such as Log P, P k_a and depiction of the bio pharmaceuticals, In pharmaceutical analysis capillary electrophoresis have been applied to the determination of adulterants in food stuffs. It determines the purity of pharmaceutical compounds. This article Converse about the general theory and instrumentation of CE along with the classification of various CE techniques, CZE, Capillary MEKC CLEF, CGE. It also Confer an overview of research on the applications of different CE techniques in the impurity profiling of drugs in the past decade. The review briefly Introduce a comparison between CE and liquid chromatography methods and highlights the strengths of CE using drug compounds as examples. This review will help scientists, fellow researchers, and students to understand the Implementation of CE techniques in the impurity profiling of drugs.

KEYWORDS: Capillary electrophoresis, liquid chromatography.

1. INTRODUCTION

Impurity because something occurs impure/Rear something else impure. The purity of substances is turn committed by the basis of the % of the labelled amount of API found by acceptable analytical method. Nevertheless, a drug substance may be considered as compromised with account to purity even it contains an impurity with superior pharmacological/toxicological properties. In assuring the high standard attribute of drug products reached into the market, it's important in screening impurities existing in the medicinal product all over manufacturing. Impurity is an unwanted substance present in the active pharmaceutical ingredients that form during the synthesis process of active pharmaceutical ingredients or any unwanted constituent that is produced besides the active ingredient during the formulation or the aging of active pharmaceutical ingredients. Even the insignificant quota of impurity existing in the medicinal product may harm the patient life and compromises the purity and superiority of the medicinal product. According to international council for Harmonization guidelines, analytical monitoring of impurity is a prerequisite and mandatory requirement for approval of market authorization of the new drug substance. Any pharmaceutical product would be accomplished to serve their intended therapeutic activity when they are free from impurity. Consequently, an impurity existing in an active pharmaceutical ingredient needs to be identified and aggregate with the help of modern analytical approaches. There are many methods of impurity profiling, such as nuclear magnetic resonance (NMR), mass spectroscopy (MS), high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), and other hyphenated techniques, such as liquid chromatography-mass spectroscopy (LC-MS), gas chromatography-mass spectrometry (GC-MS), and capillary electrophoresis–mass Spectrometry (CE-MS). It is very important to assure the quality and purity of any pharmaceutical dosage form for the wellbeing of patients. The purity of any drug is based on the percentage of the amount of the labeled active pharmaceutical ingredient present in the pharmaceutical dosage form and approved by regulatory agencies, such as ICH.

Impurity profiling in pharmaceutical API

Pharmaceuticals are the compounds manufactured for use as medicinal drugs. The bulk API producing industry of well determined quality forms a essential part of all formulation related pharmaceutical industries. Over the last few decades much attention is paid with regarding the qualityof pharmaceuticals that enter the market. Due to the implementation of strict regulations by regulatory bodies, there continues an increase in product recalls from the market. One of the peak reasons for product recall is presence of impurities or degradation products

(DPs) beyond the allowed standard limits. There are two factors which determine safety of pharmaceutical drug.
i) pharmacological to toxicological ratio of drug.
ii) Impact of impurities present in drug on humans

The result of degradation of drug substance by oxidation, deamination, proteolysis and many more chemical reactions . Such impurities should be identified and quantified. Impurities in various pharmaceuticals can takes place from variety of sources:
a) Impurity occur from raw materials
b) manufacturing process adopted
c) due to instabilityof the product during processing and storage and at last
d) from the atmospheric contaminantsduring storage

Impurity profiling assists in quantification and identification of impurities in pharmaceuticals and take part a crucial role in predicting and support stability and efficacy of pharmaceutical agents. Therefore none of the regulatory bodies contribute clear definition about specific aim of detection, identification and/or elucidation of the structure and quantifying inorganic, organic or even solvent residues contemporary in bulk drugs and pharmaceutical final products."

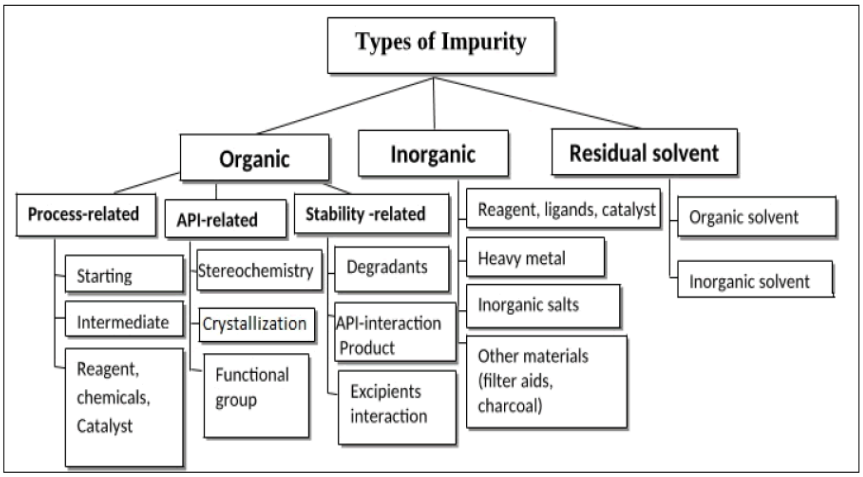
Regulatory Guidelines on Impurities in an Active Pharmaceutical ingredient

From ethical issues to competitive market besides with that mainly safety and efficacy of product supports for the impurities monitoring in the pharmaceuticals. Consequently unified regulatory guidelines are necessary that everyone has to follow to address the queries related to impurities in pharmaceuticals. USFDA have been supports the guidelines which are prepared The guidelines of the ICH has been developed by the joint effortsof the United States, European Union (EU) and Japan. ICH make certain that different NDA (NewDrug Applications) and ANDA (Abbreviated New Drug Application) application have been consistent requirements across the different regions. ICH guideline of CTD (Common Technical Document) M4Q (R1)specify in Sections 3.2.S.3.2 and 3.2.P.5.5 the requirement of characterization of impurities in new drug substances in new drug products . Guidelines of European medical agency describe the requirement of impurity enacting section CPMP/QWP/130/96 Chemistry of New Active Substances. Assessment of Quality of Medicinal Products Containing Existing or Known Active Substances.

The various regulatory guidelines regarding impurities are as follows

1 - Q1A
2 - Q3A
3 - Q3B
4 - Q3C

Types of impurities: Impurity may be divided into three types such as an organic, inorganic and residual solvent. The further classification of impurity is shown.

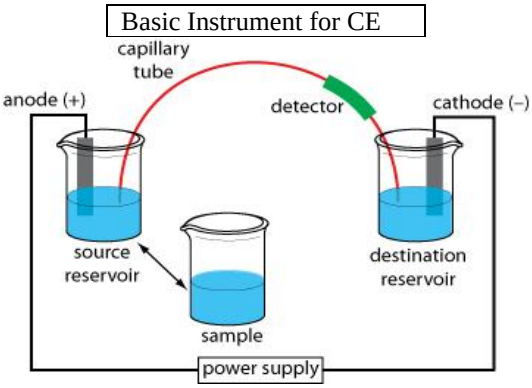


2. GENERAL INSTRUMENTATION AND METHODS OF CAPILLARY ELECTROPHOROESIS

General instrumentation of capillary electrophoresis
1) sample handling system
2) capillary catridge
3) high voltage electricity supplier
4) UV detector optics, data output and handling device.

The source vial, destination vial and capillary are filled with an electrolyte such as an aqueous buffer solution. To introduce the sample, the capillary inlet is placed into a vial containing the sample. Sample is introduced into the capillary via capillary action, pressure, siphoning, or electrokinetically, and the capillary is then returned to the source vial. The migration of the analytes is initiated by an electric field that is applied between the source and destination vials and is supplied to the electrodes by the high-voltage power supply. In the most common mode of

CE, all ions, positive or negative, are pulled through the capillary in the same direction by electroosmotic flow. The analytes separate as they migrate due to their electrophoretic mobility, and are detected near the outlet end of the capillary. The output of the detector is sent to a data output and handling device such as an integrator or computer. The data is then displayed as an electropherogram, which reports detector response as a function of time. Separated chemical compounds appear as peaks with different migration times in an electropherogram.



The Capillary Electrophoresis system consists of components that include a series of trays where the vials containing samples, buffer and other solutions were placed beyond with an interface block, a supply of high-voltage power and electrodes, temperature control hardware, a source optics module, detector and furthermore, modes of sample injection. CE separates ions using an applied voltage evolved on the electrophoretic mobility, which depends on the atomic radius, charge, and viscosity of a particular molecule. The rate about which the particles move build upon the applied electric field. As the field strength increases, the mobility of particles increases and is affected aside. The charge of the particles; Non withstanding neutral particles endure unaffected. Here, charged ions migrate under the effect of an applied electric field. If two ions of similar sizes are present, the ion with the greater charge will move faster; in the case of ions of similar charges, small-sized ions have less agitation and faster migration rates. Cations migrate facing the cathode and anions move favouring the anode. The separation of these ions is established on the electric field and remains stationary. Under normal conditions, the buffer solution moves favouring in the direction of the cathode and so do the molecules present in the solution. In CE, the sample is injected into a buffer solution present in a capillary tube. When an electric field is activated to the capillary tube, the sample components migrate because of the two types of mobility: electro-osmotic flow (EOF) and electrophoretic mobility.

Fig. 2.1: Overall view of Capillary Electrophoresis (CE) Analysis System

2.1 Electro-osmotic flow (EOF)

EOF move occupying on the function of a high voltage to an electrolyte present in a capillary tube. EOF takes place as a buffer (pH > 3) causes a proton loss from the SiOH group from a silica capillary tube to form SiO- ions The capillary wall imbibes a negative charge, which then develops a double layer of cations. The inner cation layer is stationary, whereas the outer layer is free to move along the capillary. The applied electric field details free cations to move facing the cathode, creating a powerful bulk flow. The movement of the conductive medium through a capillary in response to an applied electric field is depicted. The magnitude of the EOF can be expressed in the terms of velocity or mobility. Being the charges are strongly pH-dependent, the magnitude of the EOF varies with pH. For an effective separation, a change in the pH of the buffer plays an important role. The modification in pH from raised the isoelectric point to below the isoelectric point changes the net charge of the solute from negative to positive. An increase in buffer pH generally increases theEOF. The EOF rate can be contained by the field strength and the charge density of the capillary wall.

2.2 Electrophoretic mobility

Electrophoresis is a method in which ions migrate owing to the applied voltage. The force applied to the ions is the cumulative net charge of the products and the electric field strength. This combined expression margins to electrophoretic mobility, which is calculate of solute mobility that helps a solute to move through a conductive medium in response to an applied electric field. CE is an electrophoretic method where a buffer is added into a narrow bore silica capillary/fused silica capillary with an internal diameter of approximately (25–100 µm), and the capillary wall is encapped with ionizable silanol groups (SiOH). When the voltage is applied to the solution, molecules move through the solution facing the electrodes of opposite charges, and a detector helps to calculate the absorbance of the molecules as they pass through the

3. Types of capillary electrophoresis

The distinctive categories of CE include

a) Capillary zone electrophoresis (CZE)

b) Capillary gel electrophoresis
c) Micellar electrokinetic chromatography (MEKC)
d) Microemulsion electrokinetic chromatography (MEEKC)
e) Capillary electrochromatography
f) Capillary isoelectric focusing
g) Capillary isotachophoresis.

Electrophoresis is isolated into two major parts:
A) Continuous system
B) Discontinuous system.
The continuous system has a background electrolyte (BGE) acting throughout the capillary as a buffer and is further desperated into two subtypes: 1) the kinetic and 2) the steady-state processes. The kinetic process collect a persistent electrolyte content, whereas the steady-state process subsists of varying electrolyte content.

The sample component in a discontinuous system is categorised into two different electrolytes i.e. one with higher mobility known as leading electrolyte and other one including buffer with lowest component, which is slower than molecule.

3.1 Capillary zone electrophoresis (CZE)
CZE is greater extensively used CE method, and they were categorised to as the free solution CE technique. In CZE, the isolation is random and accessible for a mixture in solution. The principle of this mechanism is situated on the fluctate in electrophoretic mobility, which is precisely harmonious to the molecular charge and inversely equivalent to the atomic radius and viscosity of a particular solvent. The fused silica capillary acquire silanol groups, which gets ionized in the buffer solution. Anions present in the solution are aphrodisiac the anode, however a few of them migrates Tailed the cathode. Cations with the largest charge-to-mass ratio isolate first tailed by anions and finally neutral compounds. The electro-osmotic velocity can be measured by altered parameters, such as the viscosity, strength, pH, and dielectric constant.

3.2 Microemulsion electrokinetic chromatography (MEEKC)
MEEKC is a asertive method of electrokinetic chromatography that perform electrokinetic Segregation using microemulsions as background electrolytes. MEEKC separates solutes based on the hydrophobicity and distinction in electrophoretic mobility, and it assays highly efficient separation of charged and neutral solutes enclosed a wide range of water solubility. Analytes may partition interwals the aqueous phase of the microemulsion and its oil droplets, which act as a pseudo-stationary phase. This facility is well situated for the separation of neutral species containing charged oil droplets (formed by the addition of an anionic or cationic surfactant). The compounds were immiscible organic solvents (n-heptane), co-solvents (n-butanol), and surfactants behave as buffers.

Applications of capillary electrophoresis methods for impurity profiling of drugs
Capillary zone electrophoresis (CZE)
Elbashir et al. advanced and validated a CZE method for the recognition of quinocide impurities in primaquine. For evaluation purposes, native cyclodextrin and crown ether were used for the capillary ion analysis. The excutive finding by the authors were the interactions of quinocide and primaquine with buffer additives (β-CD), which led to the reverse order of elution of the analytes. The authors was able to isolate primaquine and quinocide in less than 5 min with a resolution between analytes 3.5–3.8. The authors were xplained the mechanism of complex formation and separation of analytes using buffer additives El-Attug et al. developed a mechanism to regulate kanamycin sulfate and its related impurities using the CZE technique The improvement of this method are 1) Its ability to resolve kanamycin base, its sulfate salt, and related impurities in a single run 2) It was applied to drugs lacking chromophoric groups, and separation was achieved in less than 6 min.

The authors drafted a method for the development and validation of amikacin, tobramycin, and its related impurities, nebramine and niamine, using CZE with the help of a P/ACE MDQ instrument (Beckman Coulter, USA).

A similar approach was applied and used for kanamycin, and a method was developed for drugs Journal method is that it can separate four analytes of interest in less than 5 min. The authors used the QbD access for the and utilization of this method and claimed it to be a green technology compared to the conventional analytical methods. The CZE analytical method was used for the development and validation of cinacalcet and its impurities using the QbD approach and HP 3D CE (Agilent technology) equipped with UV-Vis DAD. The analytes were isolated within 10 min CZE is a powerful separation technique employed for the impurity profiling of various drugs. Various researchers have been applied this technique for the impurity profiling of drugs, such as primaquine, kanamycin, amikacin, metformin, levosulpiride, ambrisentan, tipranavir, zofenopril, and cinacalcet. The technique was able to detect impurities below the reporting threshold according to the ICH Q3A and Q3B guidelines.

Microemulsion electrokinetic chromatography (MEEKC) Orlandini et al. used the MEEKC method with the QbD approach to determine clemastine and its three main impurities using an Agilent Technologies 3DCE system equipped with a UV-visible detector and thermosetting system microemulsion. This dual CD system-modified MEEKC method was reported for the first time as an analytical method for the analysis of clemastine and its impurities. Wongwan et al. reported a modified cyclodextrin MEEKC method for the estimation of charged and uncharged impurities of

dexamphetamine, mainly levorotary enantiomer in dexamphetamine sulfate Separation was performed using a Beckman Coulter P/ACE system. The percentage of levoamphetamine in dexamphetamine ranged from 3.2–3.8%. The method was able to quantify impurities at the 0.1% level, except for the Z-stereoisomer. The method was claimed to be the first reported CD-modified MEEKC method for the simultaneous estimation of charged and uncharged impurities, including stereoisomers.

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