Chromatographic techniques in analysis of cyclooxygenase-2 inhibitors in drugs and biological samples

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Abstract: Non-steroidal anti-inflammatory drugs, as a therapeutic class, are among the most often used active pharmaceutical ingredients in heath care in the world. They are mostly available without prescription and often used for treatment of fever and pain. An extensive research of the literature published in analytical and pharmaceutical chemistry journals has been conducted and the chromatographic methods which were used for the purity, stability and pharmacokinetic studies of the cyclooxygenase-2 inhibitors, in formulations and biological materials have been reviewed. The methodology for the analysis of selected drugs is very well documented and many examples are available in the literature. The common use of chromatographic techniques with various detection attachments provide possibility for monitoring of drugs in therapy.

Keywords: COX-2 inhibitors • Analysis • Chromatography • Stability • Pharmacokinetic

1. Introduction

Chromatography is a simpler and more accurate technique to perform qualitative and quantitative developments of the individual components than separated directly from the mixture. The basics of chromatography were defined by Tswett [1] over 100 years ago. The great progress in its use was directly linked to the development and improvement of technologies that promote chemical industry which comprises pharmaceutical, biotechnology, clinical and many other. The essential technology changes include higher pressure liquid systems, more regular and smaller particle stationary phases, modified media, more repeatable sample introduction systems, and a wide range of detectors much more sophisticated than the visual detection. However, these innovations developed the chromatography systems needed significant technology infrastructure to preserve them, including part inventories and skilled technicians to perform repairs, preventive maintenance, and system updates. These more advanced systems in many instances have introduced significant development [2].

Because of the very nature of requirement of separation of multiple components during analysis of stability samples, chromatographic methods have taken precedence over the conventional methods of analysis. Apart from separation of multiple components, the advantage of chromatographic methods is that these possess greater accuracy and sensitivity for even small quantities of degradation products produced. Most viewed chromatographic methods that have been used are thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), and gas chromatography (GC). A large number of publications have appeared in the last decade on the use of LC for stability-indicating method development.

Non-steroidal anti-inflammatory drugs (NSAIDs) are pharmaceuticals which have been used in treatment with analgesic, antipyretic and anti-inflammatory effects, and which are relatively safe. One of the causes for their popularity is that, they do not give sedation, respiratory depression or addiction. Although their chemical structure is variable all of them share the acidic nature. Recently, new drugs have been introduced, such as the second-generation cyclooxygenase (COX) inhibitors or coxibs (e.g. celecoxib).

Selective inhibitors of the cyclooxygenase-2 (COX-2), also are mentioned to as coxibs, have been developed...
as substances with therapeutic actions suchlike to those of NSAIDs, but without the gastrointestinal side effects. Long-term studies tried to explore the efficiency of coxibs in preventing creation of adenomatous colon polyps or Alzheimer’s disease have shown that selective and nonselective COX inhibitors may increase the frequency of cardiac infarctions and other cardiovascular reactions [3].

Because of the elevated polarity and acidic nature of NSAIDs (pKa values 4 - 4.5), LC-MS and LC tandem MS have experienced a leap during the last years, both in the field of technological development and application, avoiding the derivatization step demanded by GC-MS methods, particularly for acidic drugs.

Chromatographic separation is another important process in reducing ion suppression. Complete chromatographic separation enhances detectability and reproducibility despite the fact that the separation is not necessary using MS/MS detection. Due to the acidic character of NSAIDs the use of buffer or acidic solutions (such as ammonium formiate or formic acid) in the eluent is preferred. It causes reduction of signal intensities due to suppressing effects in the MS interface [4].

Several chromatographic methods have been set for the studies of NSAIDs and their metabolites in miscellaneous samples. Impurity profiling is a general term including structure elucidation/identification as well as determination of the impurities of a chemical substance. The significance of this process in research has been emphasized multiple times with chromatographic techniques always being mentioned as a widely used and extremely valuable analytical tool in this field. Besides efficacy and quality, safety of the drug substances and finished drug products is the most important prerequisite in the pharmaceutical industry. Patients of different age and different stage of illness may need to take drugs for long times: therefore these products must comply with maximal standards of safety and quality. Therefore, analytical methods have to be able to conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, dry heat, etc. and separation of drug from degradation products. In available literature there are several review articles about the determination of COX-2 inhibitors in various samples [5-8]. These present all accessible analytical methods (chromatographic, spectrophotometric, electrochemical and other) that were used to determination of the active contents of selected drugs in pharmaceutical and biological samples, and were validated according to the current procedures. Sample preparation, method’s conditions and detections were discussed.

The aim of this review is to present published work on the application only of chromatographic procedures for the analysis of coxibs (Fig. 1), covering an extended period from 2000 to present. The procedures are reviewed in terms of sampling strategies, stability, determination of metabolites, and pharmacokinetic studies. The article provides a complementary approach for pharmacokinetic studies that demand a chromatographic method of analysis for the temporal and quantitative study of the parent drug, as well as the metabolite species in a biological matrix. The analytical parameters and methods of sample preparation used in the discussed LC methods (such as sample type, stationary phase, mobile phase, detection) are presented in Table 1.

2. Chromatographic techniques in coxibs researches

2.1. Purity and stability studies

Purity and stability testing is an important part of the process of drug product development. Chromatographic methods were very often developed to separate impurities and degradates from the drug substance to monitor its quality and establish a stability profile. Stress testing of the drug substance, which is carried out under thermal, humid, oxidative, acidic, alkaline, and photolytic conditions, helps to determine the intrinsic stability of the compound.

2.1.1. Celecoxib

The identification of the five impurities present in crude celecoxib drug was described. Impurities I (4-methylacetophenone) and II (methyl-4-methylbenzoate) were characterized by LC-MS data and further confirmed by synthesis. Impurities III (5-(4-methylphenyl)-3-trifluoromethyl-1H-pyrazole), IV (4-[5-(2’-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide) and V (4-[4-(4’-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]-benzenesulfonamide) were isolated by preparative reversed phase high-performance liquid chromatography (RP-HPLC) method and were characterized using spectral data [9].

A RP-HPLC method was developed and validated for forced-degradation study. Separation was carried out on RP C-18 column with buffer: acetonitrile (40:60, v/v) as mobile phase, and monitored on photo-diode array detector at a wavelength of 254 nm. It will be useful to determine assay and known impurity of celecoxib [10].

A TLC method for quantitation of doxazosin mezylate and celecoxib in capsules, in the presence of their degradation products was described. TLC silica gel GF254 and cyclohexane: dichloromethane: diethylamine (50:40:10, v/v/v) were used as a stationary and mobile