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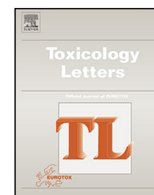

Determination of the impurities in drug products containing montelukast and in silico/in vitro genotoxicological assessments of sulfoxide impurity

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HIGHLIGHTS

- Several impurities in commercial drugs containing montelukast were quantified.
- Sulfoxide impurity was higher than qualification threshold in some drugs.
- Qualification for sulfoxide impurity was done using in silico/in vitro tests.
- Sulfoxide impurity was dose-dependent cytotoxic in human peripheral lymphocytes.
- Sulfoxide impurity was concluded to be classified as a nonmutagenic impurity.

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ABSTRACT

Impurities affecting safety, efficacy, and quality of pharmaceuticals are of increasing concern for regulatory agencies and pharmaceutical industries, since genotoxic impurities are understood to play important role in carcinogenesis. The study aimed to analyse impurities of montelukast chronically used in asthma theraphy and perform genotoxicological assessment considering regulatory approaches.

Impurities (sulfoxide, cis-isomer, Michael adducts-I&II, methylketone, methylstyrene) were quantified using RP-HPLC analysis on commercial products available in Turkish market. For sulfoxide impurity, having no toxicity data and found to be above the qualification limit, in silico mutagenicity prediction analysis, miniaturized bacterial gene mutation test, mitotic index determination and in vitro chromosomal aberration test w/wo metabolic activation system were conducted.

In the analysis of different batches of 20 commercial drug products from 11 companies, only sulfoxide impurity exceeded qualification limit in pediatric tablets from 2 companies and in adult tablets from 7 companies. Leadscope and ToxTree programs predicted sulfoxide impurity as nonmutagenic. It was also found to be nonmutagenic in Ames MPF Penta I assay. Sulfoxide impurity was dose-dependent cytotoxic in human peripheral lymphocytes, however, it was found to be nongenotoxic. It was concluded that sulfoxide impurity should be considered as nonmutagenic and can be classified as ordinary impurity according to guidelines.

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1. Introduction

An impurity is any component of drug substance or product that is not the chemical entity defined as drug substance or as an excipient of the drug product (ICH, 2006a,b). The presence of impurities in drugs may influence the efficacy and safety of pharmaceutical products even in very small amounts and aside from no benefits; they only convey health risks (Pilaniya et al., 2010). Genotoxic impurities which can induce genetic mutations, chromosomal breaks and/or chromosomal rearrangements have a special place within the context due to the fact that they bear the potential to cause cancer in humans who are treated with pharmaceuticals that contain these impurities (Raman et al., 2011). It is estimated that in the intermediary steps within pharmaceutical development process drug product may have 20–25% potentially genotoxic intermediates (Delaney, 2007).

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In recent years, a number of reviews were published on impurities (Muller et al., 2006; Delaney, 2007; Giordani et al., 2011; Dobo et al., 2006) and several regulations related to impurities in drug applications have been introduced by international regulatory authorities such as EMA (European Medicines Agency), FDA (Food and Drug Administration) and ICH (International Conference on Harmonization) (EMA, 2006; FDA-CDER Guideline, 2008a; ICH, 2006a,b; ICH, 2011). Although the efforts focus on eliminating impurities from drug substance or drug products, technically it is often not possible to remove all impurities and the elimination processes also increase the costs. Therefore, in controlling impurities, an acceptable risk level concept or threshold of toxicological concern (TTC) approach is recommended (Bercu et al., 2008; EMA, 2006). Studies and debates on impurities are continued by regulatory bodies, specialists, stakeholders and likewise regulatory approaches are dynamically furthered as well. Although authorities concentrate on guidance on new drug substances and drug products during their clinical development and subsequent applications for marketing, they recommend relevant studies to be carried out in some circumstances on new marketing applications and post approval submissions for marketed products (ICH, 2014).

The montelukast from leukotrien antagonists is commonly used for controlling asthma that is one of the most common chronic respiratory disorders in people of all ages in all parts of the world (The Global Asthma Network's Report, 2014; U.S. NIH, Asthma Report, 2007) and its six organic impurities were mentioned in American and European Pharmacopeia in 2010. Since asthma typically begins much earlier in life than other chronic diseases and lasts quite a long time, use of medication in asthma requires more attention. Furthermore, probable chronic impurity exposures should be scrutinized thoroughly to avoid unnecessary exposures, particularly in children.

This study aimed to quantify the specified impurities in drug products containing montelukast as an active substance collected from pharmacies in Turkey and to assess mutagenic/genotoxic activity of the impurities which exceed the qualification threshold according to guidelines. In this context, the following objectives were set to be achieved; (a) to quantify six impurities which have been specified in USP (American Pharmacopeia) in montelukast containing drug products available in the Turkish market for adults and pediatric use, (b) to assess the impurities of each drug product according to guidelines on impurities in terms of reporting, identification and qualification thresholds, (c) to assess genotoxicological potential of the impurity which exceed the qualification threshold, respectively by performing in silico structure activity relationship analysis, bacterial reverse mutation test (Ames test), mitotic index determination and in vitro chromosomal aberration test, and (d) to classify these impurities.

2. Material and methods

2.1. The analysis of impurities in drug products using RP-HPLC

Quantitation of 6 impurities (sulfoxide, cis isomer, michael adducts I&II, methylketone, methylstyrene impurities) already indicated in USP was conducted using RP-HPLC analysis in two batches of 20 commercial drugs containing montelukast in Turkish market (4 mg for children; 10 mg for adults) from 11 pharmaceutical companies. Instead of providing generic names, drugs were coded with letters in lieu of the relevant pharmaceutical companies, and doses and drug forms of these drugs were also specified.

2.1.1. HPLC analysis

An analytical method for montelukast impurities was developed by modification of available methods in the literature (Saravanan et al., 2008; Sivri, 2009; Goverdhana et al., 2009) and it was validated according to ICH guideline (Table 1) (ICH, 2005). Montelukast sodium (CAS#:151767-02-1), sulfoxide impurity (CAS#:909849-96-3), cis isomer impurity (CAS#:774538-96-4), methylketone impurity (CAS#:937275-23-5), methylstyrene impurity (CAS#:918972-54-0), michael adducts I&II impurity (CAS#:1187586-61-3, 1187586-58-8) standards were supplied by Molcan Corp., Canada (Fig. 1). Acetonitrile, methanol, ortophosphoric acid, sodium dihydrogene phosphate, and water were purchased from Merck. 5-Methyl 2-nitrophenol (Sigma) was used as an internal standard. Michael adducts I & II impurities' standard was provided as a mixture of diastereomers. These impurities were not resolved entirely by the method; however analyses were done by considering total peak areas of two isomers as it has been refered in the USP.

Analyses were performed on a Shimadzu Prominence LC-20A HPLC system which consists of degasser (DGU-20A3), pump (LC-20AD), photodiode array detector (SPD-M20A), and the column oven (CTO-10AS). Chromatographic analyses were performed on a C18 analytical column (150 mm × 4.6 mm, 5 μm, Kromasil). The HPLC system was controlled using LC solutions, ver. 1.25 PC software (Shimadzu Corporation, Kyoto, Japan). The column temperature was maintained at 27 °C and the PDA detector was set at wavelength of 225 nm. The injection volume was 20 μl. Mobile phases consisted of solution A: 12.5 mM sodium dihydrogen phosphate (buffer pH 3.7 adjusted with diluted ortophosphoric acid) and acetonitrile in the ratio 63:37 (v/v), and solution B: a mixture of acetonitrile and water in the ratio 90:10 (v/v) at a flow rate of 1.1 ml/min. The gradient elution program was as follows: 50% solution A for 2 min, 13 min a linear decrease from 50% to 10% solution A, for 8 min 10% solution A maintained, then followed by a linear increase from 10% to 50% solution A in 3 min.

Preparation of montelukast samples from drug products: 10 tablets/chewing tablets/granules were weighed and grinded for

Table 1
Validation results for the RP-HPLC method.

	Sulfoxide	Michael adducts I&II	*Cis* isomer	Methylketone	Methylstyrene	Montelukast
Specificity (relative retention time, min.)	0.31	0.59/0.61	0.80	1.06	1.84	1
Linearity	$y = 0.881x - 0.047$ $R = 0.9996$, $r^2 = 0.9991$	$y = 0.790x + 0.036$ $R = 0.9995$, $r^2 = 0.9990$	$y = 1.427x - 0.018$ $R = 0.9993$, $r^2 = 0.9987$	$y = 1.155x - 0.065$ $R = 0.9990$, $r^2 = 0.9980$	$y = 0.676x - 0.024$ $R = 0.9990$, $r^2 = 0.9980$	$y = 1.315x + 0.195$ $R = 0.9995$, $r^2 = 0.9990$
Accuracy and recovery (mean recovery%, RSD%)	99.68, 2.40	101.31, 0.33	106.23, 2.69	106.56, 1.33	101.27, 2.82	106.93, 2.59
Intra-day precision (RSD%)	2.36	3.46	3.92	4.00	3.52	1.77
Inter-day precision ($n = 3$ day; RSD%)	0.74 – 2.23	0.37 – 3.10	0.72 – 1.00	0.55 – 2.50	1.72 – 3.41	0.20 – 1.40
Limit of detection (μg/ml)	0.04	0.08	0.06	0.09	0.07	0.03
Limit of quantification (μg/ml)	0.13	0.23	0.18	0.26	0.21	0.09

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