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Isotopic ratios to detect infringements of patents or proprietary processes of pharmaceuticals: Two case studies

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ABSTRACT

Because of the increasing problem of drug counterfeiting and the potential danger related as well as the economic losses involved, the pharmaceutical industry and the regulatory instances are interested in the development of anti-counterfeiting and patent protection methodologies. In this paper, the evaluation of measured isotopic ratios by means of explorative chemometric techniques was performed to distinguish groups in two data sets containing samples of acetyl salicylic acid and ibuprofen, respectively. The samples in the data sets originated from different countries and manufacturers. For both compounds a clear distinction of groups of samples could be obtained. These groups could be explained based on the origin of the samples, both geographically as well as based on the manufacturer. Hypotheses were formulated concerning the synthetic pathways of the molecules and they were linked to the groups obtained with the chemometric tools.

1. Introduction

Counterfeit medicinal products are defined by the World Health Organisation (WHO) as products "deliberately and fraudulently mislabelled with respect to identity and/or source" [1]. This comprises products with wrong quantitative and/or qualitative composition, without active substances, with possible toxic substances or with correct composition in fake packaging [1].

Until recently, counterfeit drugs were only a problem of developing countries, due to a weak drug regulatory control and enforcement together with some other factors, such as scarcity, erratic supply of basic medicines, uncontrolled distribution chains, large price differences between genuine and counterfeit medicines, and lack of effective intellectual property right protection. However, counterfeiting products are increasingly becoming a serious global problem into the European and North American markets. This is possible through the bulk imports of raw materials or the bulk purchase of pills from abroad, but also through the rising popularity of so-called internet pharmacies, often selling counterfeit products originating from different developing countries [1,2].

Counterfeit products not only represent a financial drain for the pharmaceutical industry, but they are also a serious threat to public health, due to the lower quality of active substances, if at all present, excipients and the possible presence of toxic substances. The result of a counterfeit use could be that the medication is not effective or could lead to a long-term disease or injury [3–5]. Therefore, both the pharmaceutical industries and the regulatory instances are interested in the development of reliable methodologies of patent protection and anti-counterfeiting [6,7].

Most of the methodologies presented for the fight against counterfeiting of drugs are based on the determination and identification of trace impurities. In 1992, the Food and Drug Administration issued a report in which their approach in uncovering fraud in the generic drug industry was described [1]. The report focuses on the analysis of the excipients rather than the active ingredients, and the techniques used included Fourier-transform infrared spectrometry, thermogravimetric analysis, and liquid chromatography (LC) and gas chromatography (GC) [1]. In recent years, chromatographic techniques have become the methods of choice for obtaining drug impurity data. This is mostly due

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to considerable technological progress, contributing both to robustness and practicability. Also the use of hyphenated techniques such as GC-mass spectrometry, have contributed to the popularity of chromatographic techniques in this domain.

These chromatographic techniques are usually applied to identify specific compounds that can be linked to side reactions in the synthetic process of the active substance or to one of the excipients used [8]. A fingerprinting approach using pattern recognition on a part or the complete chromatogram of trace organic impurities of pharmaceuticals of different manufacturers has also been reported useful [2,9]. To identify counterfeit drugs also the use of near infrared spectrometry combined with multivariate modeling and classification is described in the literature [10].

However, these different approaches only lead to indirect evidence of fraud in the chemical and formulation processes, and are unable to screen the active substance itself. Stable isotope analysis, that probes into the atomic composition of the molecules themselves, has now appeared as a valuable and complementary technique for a number of applications in the pharmaceutical industry.

Stable isotopes are naturally occurring chemical tracers that are generally available in measurable concentrations. They are determined by isotope ratio mass spectrometry (IRMS) or by nuclear magnetic resonance (NMR) spectroscopy. These techniques are often used in the analysis of food and beverages. Repeatability and reproducibility of the data have been determined by several interlaboratory comparison studies, e.g. for ^{2}H and ^{18}O in fruit and vegetables by IRMS [11], for ^{2}H in fruit juices by NMR [12] and for ^{13}C in sugars and pulp from fruit juices by IRMS [13]. In a similar way to food products, where stable isotope content is used to differentiate between botanical origins, and between natural and synthetic sources, similar information can be obtained on pharmaceutical products. Compounds-specific isotopic information can provide an indication of the source of the raw material used in a reaction, and can help distinguish between two products that have been manufactured in different ways.

The goal of this study is to verify whether the isotopic ratios can be used as markers in order to identify counterfeiting and violations of the patented drugs production. This research was part of the EU-Counterpharm project. The idea is that stable isotope composition of the active molecule of a drug, determined either as an overall ratio by IRMS or as an isotopomeric profile obtained by NMR gives data, that is impossible to falsify for both compound and process recognition. Isotopic ratios were measured for two commonly used and commercially accessible drugs, i.e. ibuprofen and acetyl salicylic acid. Samples were purchased from different countries and manufacturers. Different chemometric tools were applied, i.e. principal component analysis (PCA) [14–16], projection pursuit (PP) [16] and multiple factor analysis (MFA) [17,18], in order to find patterns in the experimental data that could be linked to the origin and/or the manufacturing process of the drugs.

2. Theory

2.1. Synthetic pathways

For acetyl salicylic acid there is only one synthetic route, involving the acetylation of salicylic acid, obtained through carboxylation of phenol. Fig. 1 represents the synthetic pathway of acetyl salicylic acid.

Four different synthetic routes can be distinguished for ibuprofen. Fig. 2 shows the schematic representations for the different syntheses of ibuprofen.

2.2. PCA

PCA is a projection method [14,15]. It allows projecting high dimensional data into a low-dimensional space of new variables called principal components (PCs). Principal components are orthogonal and are constructed as linear combinations of the explanatory variables to maximise the description of the data variance. The projections of objects onto PCs are scores, and the projections of variables onto PCs are loadings. Therefore, scores inform about similarities among objects, while the loadings show the contributions of the different variables to a given PC and the correlation among the explanatory variables [14,15].

2.3. PP

PP is also a projection technique [15,16,19,20]. Similar to PCA, with PP the high-dimensional data are projected onto a low-dimensional space spanned by a few latent factors, called projection pursuit features (PPFs). Contrary to PCA, PPFs are obtained by maximising the projection index describing inhomogeneity of the data. Thus, with PP, a better insight into the data structure can be achieved and groups of similar samples that are not observed by PCA can be revealed. Eventually such groups of samples, if they exist, might be related to similar synthesis pathways. Therefore the kurtosis projection [21–23] index was chosen among the different projection indices available [20,22–24]. To obtain the PPFs, the Croux and Ruiz-Gazen algorithm [21] was used. In the first step of this algorithm, the data are sphered attributing to all variables the same importance in the further analysis (each variable has mean equal to zero and unit variance). After all objects are projected onto the normalized directions defined by the data origin

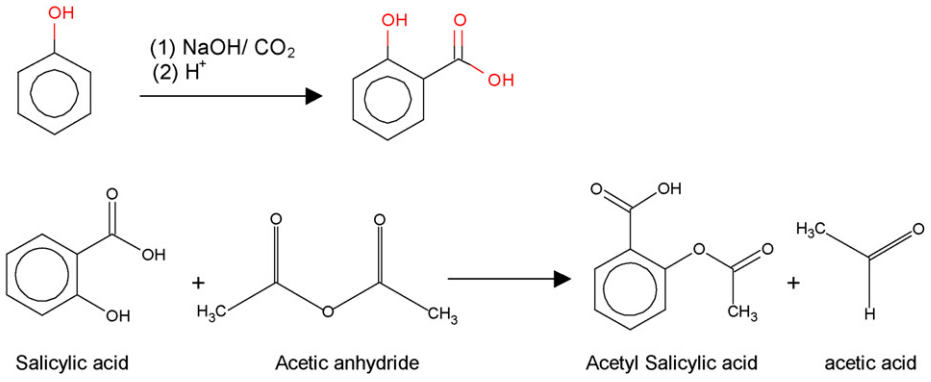

Fig. 1. Synthetic pathway for acetyl salicylic acid.