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Development of a Stationary Phase Optimised Selectivity Liquid Chromatography based screening method for adulterations of food supplements for the treatment of pain

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ABSTRACT

Illegally adulterated dietary supplements are an increasing problem worldwide. One of the important groups of often adulterated products are the dietary supplements, sold for the treatment of pain. These often contain analgesics, a heterogeneous group of molecules, containing both hydrophilic and hydrophobic compounds. The development of a screening method for these components, especially when mass spectrometric detection is not available, necessitates chromatographic separation, difficult to achieve with traditional chromatographic columns.

In this paper Stationary Phase Optimised Selectivity Liquid Chromatography was used for the development of a screening method for nine analgesics, codeine and caffeine, often present in this type of dietary supplements. The method shows a good separation of all the compounds, allowing the screening to be performed with diode array detection and is fully compatible with mass spectrometry. The method was validated for its selectivity following the guidelines as described for the screening of pesticide residues and residues of veterinary medicines in food.

1. Introduction

The distribution of counterfeited and substandard medicines is an increasing problem worldwide. In developing countries it is estimated that about 30% of the medicine market is covered by counterfeit medicines. Here not only essentially lifesaving medicines like antibiotics, anti-malarial products, HIV inhibitors, etc. are counterfeited, resulting in higher mortalities, but also in resistance of certain viruses and bacteria, due to inadequate treatment. In industrialised countries less than 1% of the medicine market is affected. In these countries mostly counterfeited life style drugs are encountered. Sometimes some counterfeited antibiotics or other essential drugs were detected in both the United States as in the European Union [1–4]. Another increasing problem is the availability of adulterated dietary supplements and traditional medicines [1]. Dietary supplements and traditional medicines are now freely available through internet or in some special shops. Care should be advised, since these products, especially when they are sold by internet sites, disclosing their identity or other illegal suppliers, are often adulterated with pharmaceutical ingredients. Essentially four important indication groups of adulterated dietary supplements can be distinguished: slimming, potency enhancement, muscle building and treatment of pain [1,5]. This paper will focus on the latter group. Dietary supplements for the treatment of pain, sold through an illegal supply chain, often claim to be of herbal nature or to contain 100% natural compounds, but are in fact adulterated with analgesics. The group of analgesic medicines is constituted of a heterogeneous group of molecules with a wide variety of molecular structures and log *P* values. This makes it difficult to develop a screening approach with both good separations for the more hydrophilic compounds and good separation and detection of the more hydrophobic molecules. Even if detection with mass spectrometry does not necessitate separation, it is recommended for a screening method to have a certain separation of the molecules. The more, not all laboratories confronted with the analysis of adulterated dietary supplements are equipped with mass spectrometry and therefore a screening method allowing good separation and identification based on retention time and diode array data is advantageous.

Stationary Phase Optimised Selectivity Liquid Chromatography or SOS-LC is a relatively unexplored technique to solve separations in which both groups of hydrophilic as well as groups of hydrophobic molecules have to be separated and detected in the same run. In SOS-LC the stationary phase becomes a tunable parameter by serial connexion of column segments, with variable lengths, of different stationary phases [6]. The basic development kit sold by Bischoff

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chromatography (Bischoff Analysentechnik u. geraete GmbH, Leonburg, Germany) consists of segments of 1, 2, 4, 6 and 8 cm of five different stationary phases, i.e. ProntoSIL C18 SH-2, ProntoSIL C18 EPS-2, ProntoSIL Phenyl-2, ProntoSIL CN-2 and ProntoSIL C30 [7]. The basic idea is to run samples, containing the molecules of interest on the different stationary phases separately and based on the retention times obtained, calculate the optimal combination of segments for the separation. In the literature only a few applications using SOS-LC are described. Zedda et al. [8] proposed a liquid chromatographic screening method with mass spectrometric detection, based on SOS-LC, for polymer electrolyte membrane degradation products. Keuhnle et al. [9] and De Beer et al. [6] both presented SOS-LC methods for the separation and the analysis of different steroids and Chen et al. [10] presented the potential of SOS-LC in the domain of green chemistry. All these applications are focussed on the targeted screening of a series of molecules and this in an as short as possible run. The situation of laboratories, charged with the screening of suspected dietary supplements is however different. Often a set of molecules belonging to a therapeutic class is selected for the development of the screening method. The separation is optimised, but the run is kept quite long in order to ensure the detection of other (possibly unknown) molecules during the run. This is necessary since a large variety of molecules, possibly of a total different therapeutic class as the one suggested by the indication, might be present as well as designer molecules. The advantage of SOS-LC in the development of screening methods is the fact that both the stationary as well as the mobile phase can be adapted to obtain the best separation/detection of the compounds of interest. The basic idea of SOS-LC is that you keep the mobile phase composition constant and the stationary phase is adapted by changing type and length of the column segments in order to achieve the optimal separation. The idea in the presented approach is that both stationary phase and mobile phase can be adapted in order to obtain good separation of a set of molecules that are hard to separate using a classic chromatographic column.

In this paper a screening method using SOS-LC for dietary supplements with the treatment of pain as indication was developed. Therefore retention times for a set of 10 often found adulterants were measured isocratically on the five stationary phases of the Bischoff development kit. Caffeine was used as the 11th component since it is present in the majority of the dietary supplements for the treatment of pain analysed in our laboratory. Including caffeine in the development/validation set ensure that the presence of caffeine will not interfere with the screening. Based on isocratically measured retention times the optimal stationary phase was calculated and used for gradient optimisation. After validation the method was applied for the screening of five samples seized by the Belgian authorities.

2. Methods and materials

2.1. Chemicals and reagents

The reference standards for paracetamol, acetyl salicylic acid, aminopyrine, caffeine, phenylbutazone, and ibuprofen were purchased from Fagron (Waregem, Belgium). Ketoprofen, naproxen, aceclofenac, diclofenac sodium salt and indomethacin were purchased from Sigma-Aldrich (St. Louis, USA) and Codein Phosphate Hemihydrate from Conforma (Destelbergen, Belgium). Uracil for determination of death volumes was purchased from Across (Geel, Belgium).

For the preparation of the mobile phases ammonium acetate, glacial acetic acid and formic acid were purchased from Merck (Darmstadt, Germany) and methanol, HPLC-grade, from Biosolve (Valkenswaard, The Netherlands).

Lactose and *sirupus simplex* used as blank matrices were purchased from Fagron (Waregem, Belgium). The blank herbal matrices used for the validation were all herbal dietary supplements, previously analysed at our laboratory and found negative for active pharmaceutical substances. All these samples were donated by the Federal Agency for Medicines and Health Care Products (FAMHP) of Belgium. Also the five real samples, to which the method was applied, were kindly donated by the FAMHP. The samples were seized by their inspectors. All these samples were confiscated at customs, due to doubts about the origin of the products. All the five samples claim to be composed of 100% herbal compounds and were probably bought by individuals through internet.

2.2. Instrumental conditions

Method development and validation was performed on a Waters 2695 Alliance® chromatographic system (Waters Corporation, Milford, USA). The system consisted of a quaternary pump, a temperature controlled autosampler and a column heater, coupled to a Waters® 2998 Diode Array Detector (DAD). The output signal was monitored and processed using the Waters Empower3 software. The SOS-LC method was developed using a POP-LC basic kit 250-5 (Bischoff Analysentechnik u. geraete GmbH, Leonburg, Germany). This kit consists of one segment of 1, 2, 6 and 8 cm and 2 segments of 4 cm for five different stationary phases, i.e. ProntoSIL C18 SH-2, ProntoSIL C18 EPS-2, ProntoSIL Phenyl-2, ProntoSIL CN-2 and ProntoSIL C30 [7]. The kit also contains the necessary holder segments and inlet and outlet filters as well as the POPLC optimizer software for isocratic runs.

The primary runs used for the calculation of the optimal stationary phase were performed on columns consisting of 20 cm of each of the 5 types of phases separately. These primary runs were run in isocratic mode with a mobile phase consisting of 50% of an aqueous 0.025 M ammonium acetate buffer of pH 4 and 50% of methanol. Flow was 0.5 ml/min, column temperature was set at 27 °C, injection volume was 5 μl and the molecules were detected at a wavelength of 254 nm.

The retention times obtained for each of the molecules were used for the calculation of the optimal stationary phase, used for gradient optimisation in order to obtain the desired screening method.

Validation was performed on the optimised column consisting of a 10 cm segment of ProntoSIL C18 EPS-2 and an 8 cm segment of ProntoSIL CN-2. The mobile phases consisted of the previously mentioned ammonium acetate buffer and methanol. The optimal gradient for the screening method started at 1% organic modifier, which was held for 15 min. Afterwards a linear gradient was started going to 40% of organic modifier in 10 min, followed by a linear gradient to 60% of organic modifier in 25 min. This plateau was held for 10 min before returning to the initial conditions. The flow was 0.7 ml/min, column temperature was 27 °C, injection volume was 5 μl and the detection wavelength was set at 254 nm.

2.3. Sample preparation

2.3.1. Standards

For paracetamol, aminopyrine, caffeine, ketoprofen, naproxen, phenylbutazone, aceclofenac and diclofenac a solution of 0.1 mg/ml of each compound separately was prepared in methanol. For codeine phosphate hemihydrate and ibuprofen a solution of 0.3 mg/ml in methanol was prepared and for acetyl salicylic acid a solution of 0.3 mg/ml was prepared in methanol containing 1% of formic acid. The latter to guarantee the stability of acetyl salicylic acid.

Next to the solutions containing each compound separately a mixture was prepared with the same concentrations of each molecule in methanol containing 1% of formic acid.