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Development of a method for the analysis of seven banned azo-dyes in chilli and hot chilli food samples by pressurised liquid extraction and liquid chromatography with electrospray ionization-tandem mass spectrometry

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ABSTRACT

An automated, confirmatory and sensitive procedure has been developed and validated for the determination of Sudan (I–IV), Sudan Orange G, Sudan Red 7B and Para Red in hot chilli food samples. The proposed method includes pressurised liquid extraction (PLE) with acetone, gel permeation chromatography (GPC) clean-up and detection by liquid chromatography (LC) coupled to electrospray ionization in positive mode tandem mass spectrometry (ESI-MS–MS). The main parameters affecting the performance of the different ionization sources and PLE parameters were previously optimised using statistical design of experiments (DOE). The method was in-house validated on chilli powder and chilli meat. Linear calibrations were obtained with correlation coefficients $R^2 > 0.999$. The limits of detection (LOD) and quantification (LOQ) of the method were in the ranges of 0.002–0.012 ng g^{-1} and 0.006–0.036 ng g^{-1}, respectively for chilli powder. The decision limit and detection capability were between 0.005–0.022 ng g^{-1} and 0.007–0.026 ng g^{-1}, respectively for chilli meat. Recoveries ranged from 94% to 105%. The applicability of the method to the determination of azo-dyes in hot chilli products was demonstrated.

1. Introduction

Food quality is closely associated with colour and the use of food colourants has been an age-old practice, enhancing the aesthetical appeal of foods [1]. The use of synthetic organic dyes has been recognised as the most reliable and economical method of restoring or providing colour to a processed product. Azocompounds are by far the most widely used synthetic colourants [2]. Azo-dyes are synthetic organic colourants, characterised by chromophoric azo groups (—N=N—). Next to Sudan I–IV, Para Red, Butter Yellow, Sudan Red and Sudan Orange G belongs to the group of azo-dyes. Currently, there are over 3000 azo-dyes in use worldwide and they offer a wide spectrum of colours [3].

In order to prevent indiscriminate use, laws and regulations have been developed by many countries limiting types, purity, uses and amounts of authorised food dyes. The list of permitted synthetic dyes is progressively being reduced [1]. The list of authorised food colours and the maximum permitted levels in foodstuff are laid down in the annexes of Council Directive 94/36/EC [4]. Among organic colourants most of azo-dyes are recognised to be carcinogens [5]. Belonging to the azo-dye class, Sudan dyes may cause cancer to people and their presence at any level is not safe for the human [6].

Sudan I is not permitted colour under the Colours in Food Regulations [7] and is considered to be a genotoxic carcinogen [8]. Sudan II is the dimethyl derivate of Sudan I and has been tested in mice bladder implantation, resulting in a high incidence of bladder carcinomas [9]. Sudan III and Sudan IV are fat-soluble dye predominantly used for demonstrating presence of triglycerides in frozen sections. In addition, Sudan III and IV are commonly used for colouring waxes, oils and spirit varnishes. Sudan I, II, III and IV have been classified as a category 3 carcinogen by the International Agency for Research Cancer [10]. Consequently, the fraud identified by adulteration of chilli and chilli products by Sudan dyes constitutes a risk for public health. Para Red is chemically very similar to Sudan I and the Food Standards Agency (FSA) independent scientific experts have advised that it could be a genotoxic carcinogen [11].

In recent years, several processed food products, including chilli powder, curry sauce, and mustard sauce have been rejected or withdrawn by the European Union (EU) because of the presence of Sudan dyes. In particular, discovery of the azo-dye Sudan I in hot chilli and hot chilli products originating from India and marketed in the EU does not comply with the EU food safety requirements [12]. As a consequence, in June 2003, the European Union Commission

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adopted a decision on emergency measures concerning hot chilli and hot chilli products intended for human consumption [13]. On January 2004 the next emergency measures Decision 2004/92/EC were adopted by the Commission on Sudan I–IV [14].

According to the data collected through the Rapid Alert System for Food and Feed (RASFF), a sharp decrease in numbers of notifications about the fraudulent use of dyes in food has been observed since 2003. The 2006 RASFF annual report showed contamination with either Sudan I or Sudan IV or a combination of both in spices from the Region of India and Pakistan. Spice mixtures from the Russian Federation contained Sudan I, Para Red and a few time toluidine red and Sudan Red G. Few notifications on spices from Far East (Vietnam, China) showed the detection of Rhodamine B. In chilli from Nigeria, higher levels of Orange II were detected [15].

In this context, the use of banned dyes in food products exported in the European Union has led to an increasing demand for quantitative and confirmatory methods for compliance verification of these foodstuffs.

Several chromatographic methods have been proposed for the quantitative determination of azo-dyes in foodstuff, among them liquid chromatography with spectrophotometric [16] or fluorimetric detection [9]. Due to its high sensitivity, selectivity and minimal sample treatment required, liquid chromatography coupled to mass spectrometry (LC–MS) has become the preferred method for confirmatory analysis. LC methods with atmospheric pressure chemical ionization (APCI) [17] and electrospray ionization [6,12] for the identification of these substances have been developed and tandem mass spectrometry LC-APCI and [18] LC-ESI [19] combined with isotope dilution methods were described for the determination of Sudan dyes in foodstuff. A capillary liquid chromatography-electrospray tandem quadrupole orthogonal-acceleration time of flight mass spectrometry was used to accurate mass measurements for the confirmation of Sudan (I–IV) in hot chilli tomato sauce with detection limits from 0.4 to 11 $\mu g\,g^{-1}$ [20] and a LC-diode array detection-electrospray mass spectrometry was developed for simultaneous determination of water-soluble and fat-soluble synthetic colourants in foodstuff by high-performance liquid chromatography–diode array detection-electrospray mass spectrometry with detection limits from 1.25 to 5 $\mu g\,g^{-1}$ [21]. Some authors used internal standards [20,21] or standard addition techniques [22] in the analysis of Sudan dyes in order to improve their quantification.

The most frequently used extraction technique in these works is conventional solvent extraction. These procedures are laborious and time consuming. Pressurised liquid extraction has been frequently used for analysis of trace residues and contaminants in foods [23–27]. This paper describes the application of PLE for the determination of azo-dyes in foodstuffs in order to increase the speed of the extraction, the efficiency, the quantity of sample and the automatization and decrease the solvent consumption [28]. Various solvents (acetone, acetonitrile and methanol) were investigated and the dependence of the extraction yield from different parameters such as the static extraction time, pressure and oven temperature was studied.

Matrix interference from the large pigment and lipophilic compounds in foodstuffs is a serious hindrance to accurate quantification of lipophilic synthetic dyes in the foodstuffs [6], so gel permeation chromatography has been used by other authors [6,29] as clean-up technique to eliminate co-extractives molecular interferences such as fatty and wax from these kind of samples, based on the great difference in molecular size between them and the azo-dyes. In this work we developed a modified GPC method in order to purify the samples.

This study is focused on the development of an automated and sensitive method for the confirmatory detection of Sudan I, Sudan II, Sudan III, Sudan IV, Sudan Orange G, Sudan Red 7B and Para Red in hot chilli products. It entails pressurised liquid extraction of azo-dyes from the hot chilli products followed by a GPC clean-up and analysis by LC–ESI-MS–MS, using Sudan I-D5, Sudan IV-D6 and Para Red-D6 as internal standards. The sensitivity of the method was improved by using GPC clean-up and by choosing the better ion source settings and LC conditions that maximised the analytical response provided by ESI. PLE parameters and ion source settings were optimised using statistically designed experiments [30]. The optimisation of both systems requires studying a multitude of parameters and can be very tedious by the approach of changing-one-factor-at-a-time (COST). In addition, this approach does not give information on interactions between experiments, so it can miss the optimal settings when interactions exist. Statistically designed experiments such as Plackett–Burman designs (P–B) and central composite designs (CCD) [31] can help optimise these kind of analytical parameters much more efficiently and in less runs than the COST approach [32,33].

The optimised method was applied to the determination of Sudan I, Sudan II, Sudan III, Sudan IV, Sudan Orange G, Sudan Red 7B and Para Red in hot chilli products selected from local supermarkets in Valencia (Spain) between January 2005 to December 2007 and can be used in the national monitoring programs to detect the presence of these illegal dyes in the framework of the European legislation.

2. Experimental

2.1. Standards and reagents

HPLC grade ethanol, acetone, dichloromethane, acetonitrile, formic acid and ammonium formiat were supplied by Merck (Darmstadt, Germany) and ultrapure water was obtained from a Milli-Q filter system (Millipore, Bedford, MA, USA). Diatomaceous earth was from Sigma–Aldrich (Steinheim, Germany). The solvents for HPLC were filtered by 0.22 μm nylon membrane (Whatman, UK) and degassed in ultrasonic bath.

Standards of Sudan I, Sudan II, Sudan III, Sudan IV, Sudan Orange G, Sudan Red 7B and Para Red and internal standard Para Red-D6 were purchased from Dr. Ehrenstorfer GmbH (Augbsburg, Germany). The purities of all standards were more than 90%. Internal standards Sudan I-D5 and Sudan IV-D6 were obtained from Witega Laboratorien Berlin-Adlershof GmbH (Berlin, Germany). The purities of the internal standards were more than 95%.

Individual stock solutions containing 200 $\mu g\,mL^{-1}$ approximately, were prepared in dimethilsulfoxide and stored at 4 °C in the dark. Intermediate mix solution at 1 $\mu g\,mL^{-1}$ and working solutions containing 1–1000 $ng\,mL^{-1}$ approximately of each standard and 50 $ng\,mL^{-1}$ of internal standards were prepared in methanol/water (9:1) and stored at 4 °C in the dark.

Sudan I and IV reference test material FAPAS T2031 (chilli powder), was provided by CSL (Central Science Laboratory, York, UK).

Statistical data manipulation and numerical analysis of data resulting from experimental design were carried out by means of the statistical package MINITAB for Windows, Release 14 (Minitab Inc., Birmingham, UK).

2.2. Equipment

Extractions were carried out using an accelerated solvent extraction system (ASE 200, Dionex, Sunnyvale, CA, USA) equipped with 22 mL stainless steel extraction cells. The waters gel permeation chromatography clean-up system employed for the purification was integrated by a Waters 515 high-pressure liquid chromatog-