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Analytical Methods

Interference-free determination of illegal dyes in sauces and condiments by matrix solid phase dispersion (MSPD) and liquid chromatography (HPLC–DAD)

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ABSTRACT

A fast, simple and effective extraction method based on matrix solid phase dispersion (MSPD) was developed and validated for the simultaneous cleaning-up and quantitative extraction of illegal dyes (sudan I, sudan II, sudan III and sudan IV) from different sauces and condiments. Several parameters as sorbent, cleaning procedure to eliminate carothenoids and other interferences, and solvents for elution were evaluated to find the optimal MSPD conditions. The best results were obtained using a system containing washed sea sand and Florisil as sorbents and sodium sulphate as desiccant; hexane was used as defatted agent and acetonitrile as elution solvent. Quantitative analyses were performed by liquid chromatography (LC) with diode array detection (DAD). The chromatographic separation was performed on a Phenomenex Synergy Polar RP column with isocratic elution using methanol/acetonitrile/water 65/20/15, v/v/v, as the mobile phase at a flow rate of 1.0 mL min^{-1} and 30 °C of temperature. Under these conditions sudan I–IV recoveries were between 60% and 99% and relative standard deviations ranging from 2.0% to 10.0%. Limits of detection resulted five times lower than the values required by European regulations and were ranged between 0.05 and 0.09 μg g^{-1}. The applicability of this MSPD–DAD method to determine illegal sudan dyes in sauce and condiment samples was demonstrated. This method has potential to be applied using a simple instrumentation present in most analytical laboratories.

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1. Introduction

Sudan dyes are a group of compounds characterised by the presence of one or more azo groups (–N=N–) used in industrial products (printing inks, waxes, candles, oils, plastics, textiles, cleaners) and scientific applications (colouring of fuel, staining for microscopy, etc.). However, azo dyes have been questioned repeatedly, because many compounds of this family have been shown to be carcinogenic in animal experiments (EFSA, 2005; Stiborova, Martinek, Rydlova, Hodek, & Frei, 2002). The European System of Fast Food Alerts (RASFF) has reported in recent years a large number of alarms on the use of banned dyes in foods, mainly by use of spices of oriental origin, which have caused great public alarm in many countries. Sudans I–IV are the substances most frequently used in this fraud, so that European legislation prevents from 2004 the marketing and sale of foods that contain them (BfR, 2003) and has established the detection limits for sudan dyes in food materials at 0.5–1 mg kg^{-1} (Commission decision, 2005).

Food matrices are very complex, and sudans are found in very low amounts, usually less than 0.1 μg g^{-1}. Therefore, to ensure the complete absence of these dyes in food, analytical methods used in laboratories must have very low detection limits, 0.01 μg g^{-1} or less, which can only be achieved through the combination of complex purification processes and the use of expensive technologies. Carothenoids cause the main interferences in the chromatographic determination of sudans in food, since they are used as colour enhancers in many products in the form of mainly plant extracts to mimic, intensify, and prolong the appearance of natural hues. Although sudan I–IV and carothenoids have different chemical structures other properties such as polarity, solubility and absorption spectra are very similar, making difficult the determination of sudans by HPLC in food matrices, so it is always necessary to carry out a laborious sample pretreatment steps to eliminate interferences.

Different analytical methods have been reported in the literature for the determination of sudans (Rebane, Leito, Yurchenko, & Herodes, 2010), such as electrochemical (Chailapakul, Wonsawat, Siangproh, Grudpan, Zhao, & Zhu, 2008), capillary electrophoresis (Mejia, Ding, Mora, & Garcia, 2007), chemiluminescence (Chang et al., 2011) and immunoassays (Ju, Tang, Fan, & Chen, 2008), being liquid chromatography–diode array detection (LC–DAD) (Cornet, Govaert, Moens, Loco, & Degroodt, 2006; Long et al., 2011; Qi, Zeng, Wen, Liang, & Zhang, 2011; Uematsu, Ogimoto, Kabashima, Suzuki, & Ito, 2007; Yan, Wang, Qiao, & Yang, 2011) and liquid chromatography–mass spectrometry (LC–MS) (Botek, Poutska, & Hajslová,

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2007; Ferrer, Fernández-Alba, & Ferrer, 2007; Pardo, Yusá, León, & Pastor, 2009; Tateo & Bononi, 2004; Ma, Luo, Chen, Su, & Yao, 2006; Murty, Sridhara Chary, Prabhakar, Prasada Raju, & Vairamani, 2009) the preferred methods. Depending of the complexity of matrix, some pretreatment steps such as liquid–liquid extraction (LLE) (Long et al., 2011; Zacharis, Kika, Tzanavaras, Rigas, & Kyranas, 2011), solid phase extraction (SPE) (Qi et al., 2011; Liu, Hei, He, & Li, 2011; Zhao, Zhao, Liu, & Zhang, 2010; Zheng, Wu, Feng, & Huang, 2011) or even MIP–SPE (Puoci, Garreffa, Iemma, Muzzalupo, Spizzirri, & Puoci, 2005; Baggiani et al., 2009) are necessary. These are usually multi-step procedures, typically based on exhaustive extraction from the matrix into organic solvents or separated by solid phase extraction, after the elimination of proteins and lipids, and the subsequent removal of co-extracted material by several clean-up steps prior to instrumental analysis. Such determination methods are laborious, time consuming and usually involves relatively large amount of solvents, which dramatically affect the recovery of the dyes and contribute highly to the total cost of the analysis.

Matrix solid phase dispersion (MSPD) is an auxiliary technique of extraction in complex matrices. MSPD involves dispersion of the sample over a solid support, followed by a preliminary purification and the subsequent elution of the analytes with a relatively small volume of solvent (Barker, 2000). The obtained extracts are generally ready for analysis, although if necessary, they can easily been subjected to direct extra purification. The use of MSPD offers an effective alternative to traditional methods for sample preparation. MSPD has been applied to the analysis of several additives in foods (Kristenson, Ramos, & Brinkman, 2006) and recently it has been applied to the analysis of banned dyes in chilli powder (Kesiunaite, Linkeviciute, Naujalis, & Padarauskas, 2009) and egg yolk (Yan et al., 2011), obtaining satisfactory results. However, up to date MSPD has not been used for the analysis of sudan I–IV in matrices as sauces and condiments.

The aim of this work was to develop and validate a fast, easy and economical MSPD method to allow the determination of banned azo dyes such as Sudan I–IV, in various sauces and condiments by liquid chromatography with diode array detection (LC–DAD), which is a simple instrumentation available in most analytical laboratories. The effects of several parameters such as solid phase support, purification and elution solvents were investigated to find the optimal MSPD conditions. This method could be potentially applied for rapid detection of sudan dyes at LOD low enough for quantitative determination under European legislation.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade solvents used were acetonitrile and methanol, supplied by Scharlau (Barcelona, Spain); ethyl acetate, chloroform, tetrahydrofuran and *n*-hexane were supplied by Merck (Darmstadt, Germany). Anhydrous sodium sulphate was purchased from Panreac (Barcelona, Spain). All reagents used were of analytical grade or better. Ultra pure water (resistivity 18.3 MΩ cm) was obtained using a Nanopure Barnstead water system with additional reverse osmosis cartridge (Millipore Ibérica, Madrid, Spain).

Solid standards of Sudan I (97%), sudan II (90%), and sudan IV (80%) were purchased from Sigma–Aldrich (Madrid, Spain), and sudan III (80%) from Fluka (Madrid, Spain). Solid phase materials used for MSPD were C18 bonded silica from Sigma–Aldrich, washed sea sand (0.25–0.30 mm) from Panreac, Celite AFA® from Fluka, Alumina N from Merck, Florisil® RS 60–110 mesh (0.110–0.250 mm) from Carlo Erba SDS (Barcelona, Spain), and silica gel grade 0.150 mm also from Sigma–Aldrich.

2.2. Samples

Nine pickling and chutney sauces having different compositions from several regions of the world (Spain, Germany, México, Ecuador) were purchased in the local market. Samples were tomato, paprika, carrot and vegetables based picking sauces. All of them contained chilli and other spices mixed with sugar, starch, oils, paprika, vinegar, garlic, salt and others.

Sauce samples were stored in the refrigerator at 4 °C. Before processing, samples were allowed to thaw at room temperature and homogenised. Recovery experiments were performed by spiking sauce samples with the desired amount of each sudan dye.

2.3. Equipments

Analysis for this study were performed using an HP 1050 Series liquid chromatographic system (Hewlett Packard, Palo Alto, CA, USA) equipped with a quaternary pump, a column compartment, a vacuum degasser and a diode-array detector. A Synergy 4 μm Polar RP80A (15 cm × 4.6 mm i. d.) column from Phenomenex (Barcelona, Spain) was used for analytical separation. Samples were manually injected through a sample injection valve (Rheodyne Inc., Model 7725; Hewlett Packard) in which a 20 μL loop was mounted. The system was controlled by Hewlett Packard CHEMSTATION software (Hewlett Packard). A thermostatic heating block of aluminium model R8 Nanocolor Macherey–Nagel (Düren, Germany) was used to evaporate the solvent of the MSPD extracts under a stream of argon.

2.4. MSPD procedure

A sauce sample of 0.50 g spiked with the desired amounts of sudan I–IV was poured into a glass mortar with 0.50 g of sodium sulphate, 0.50 g of Florisil and 0.50 g of washed sea sand. The materials were blended with the glass pestle for 2 or 3 min, until a dry homogeneous mixture were obtained, which separates from the walls of mortar and flow easily. The mixture was packed into a 6 mL solid phase extraction glass tube containing a polyethylene frit and a filter paper disk at the bottom, thus preventing obstruction the pores of the frit and staining. The packing material was slightly compressed using a piston to a final height of 20–25 mm, and covered by a second disc of filter paper on the head of the column. A polyethylene valve located at the outlet of the SPE tube controls the flow of solvent though the column. The sample was firstly defatted using 4 mL of hexane. Then 1 mL of acetonitrile (about the dead volume of the column), was eluted with the valve open while applying a slight positive pressure on the bed by a syringe with a rubber gasket and the eluate was rejected. The analytes were eluted by adding 3 mL of acetonitrile to the column and the sample was allowed to elute dropwise by applying a slight vacuum. The eluent was collected into a tube with a screw cap and evaporated to dryness over a period of approximately 5 min under a stream of argon (outlet pressure, 1–2 bar), using an aluminium metal block thermostated to 70 °C. Finally, the obtained extracts were reconstituted with 0.5 mL of mobile phase (methanol/acetonitrile/water, 65/20/15, v/v/v) and an aliquot of 20 μL were injected into HPLC–DAD system for analytes separation and quantification. All analysed samples were prepared in duplicate.

2.5. Chromatographic analyses

Liquid chromatographic analyses were carried out under the following conditions: the mobile phase was a ternary mixture consisting of methanol, acetonitrile and water in proportions of 65/20/15, at a flow rate of 1 mL min^{-1}. The total run time was 15 min and chromatographic separation was carried out at 30 °C. Quantitative measurements of the peak areas by HPLC–DAD were carried out by

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