DETECTION VON 6 SUDAN DYES, DIMETHYL YELLOW AND PARA RED IN SPICES AND SAUCE WITH HPLC/MS/MS

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INTRODUCTION

The Sudan dyes Sudan I (CAS-Nr. 842-07-9), Sudan II (CAS-Nr. 3118-97-6), Sudan III (CAS-Nr. 85-86-9) and Sudan IV (CAS-Nr. 85-83-6) belong to the group of the Azo-dyes. According to the IARC (International Agency for Research on Cancer) Azo-dyes have been classified as group 3 of potential carcinogenic substances. At oral uptake the Azo dyes can be reduced to the partially carcinogenic mono-amines, which belong to the group 2 (IARC, BIR 2003). Due to that Sudan dyes are banned as food additives in the EU. Para Red (CAS-Nr. 6410-10-2) and Dimethyl Yellow (CAS-Nr. 60-11-7) belong to the same group 2.

In May 2003 first investigations in France showed the presence of Sudan I in chilli originating from India. Reacting on that the EU-Commission initiated control arrangements in June 2003 (2003/460/EG). After several alerts from European countries the decision was taken that all imported food contain labels proving the absence of Sudan dyes (on the 21.01.04: 2004/92/EG). Products which got tested positive have to be destroyed. Due to the related expenses a reliable testing method is inevitable.

The methods described in literature are based on the analysis of the Sudan dyes by HPLC-DAD (RASFF News 03/92), GC-MS (Schäfer et al.) and LC-MS (Winkeler et al.), as well as LCM/MS/MS (Riediker et al.). The sample preparation is partially extensive and quantification limits of >100 µg/kg cannot be achieved. However, Switzerland requires a analysis obtaining quantitation limits of 100 µg/kg (BAG 2004).

In this paper we present a method which combines an easy extraction procedure with acetoniitrile and he analysis on an API 2000TM LC/MS/MS system.

SAMPLE PREPARATION

Each sample gets weight twice and one of them is spiked with the dyes. Experiences revealed that each matrix shows a very different effect on the sensitivity of the MS/MS signals. It can even vary from sample to sample from same matrices. For this reason it was chosen to determine the recovery for each sample individually.

2 x Weighing of a Homogenized Sample
1 x Additional of Standard Solution
2 x Extraction of each Sample with Acetonitrile
Filling up to a Defined Volume
Filtration
LC/MS/MS

HPLC CONDITIONS

- Agilent 1100 System
- Column: Zorbax Eclipse XDB-C8, 150x4.6 mm, 5 µm (Agilent)
- Column Oven: 40°C
- Eluent: H₂O/ACN 70/30 (0.2% FA) in 5 min auf 95%/5, then 5 min constant
- Flow: 400 µl/min, with Split 1:1 to the MS

MS/MS CONDITIONS

Applied Biosystems API 2000TM LC/MS/MS System with TurboIonSpray® – Source (ESI).

Results with the presented method more than 750 samples of different matrices have been analyzed in routine work between 2004 and 2005. 10 % of those samples were contaminated with Sudan I, 3 % with Sudan IV and V and 7 % with Sudan I und IV. Sudan II could not be found in any sample and Sudan III just in 12 samples (1,5 %), Sudan III always occurred together with Sudan I or Sudan IV. Para Red and Dimethyl Yellow were mostly found in samples which contained as well other Sudan dyes. Maximum values of 300 mg/Kg were observed for Sudan I and 580 mg/Kg for Sudan IV. Sudan I was found from low to high concentrations in chilli and mixed spices whereas mainly lower concentrations (below 500 µg/kg) were found in sweet peppers and palm-oil (Graph 4). With other methods achieving quantitation limits of 100 µg/kg approximately 30 % of the positive sample containing Sudan I and roughly 50 % of those ones containing Sudan IV would not have been identified. The detected concentrations had been confirmed by repeated sample preparation and analysis.

LITERATURE

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