

Rapid Detection of Dimethyl Yellow Dye in Curry by Liquid Chromatography-Electrospray-Tandem Mass Spectrometry

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Abstract

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An accurate and rapid method, was devised for the identification and quantitation of dimethyl yellow dye in curry, based on liquid chromatography-tandem mass spectrometry interfaced with electrospray. Mass spectral acquisition was done in positive ion mode applying two fragmentation transitions to provide a high degree of selectivity. The extraction system provided a very high recovery (100.0% to 105.8%) and good results were obtained for the limit of detection (5 µg/kg) and limit of quantitation (16 µg/kg). The applicability of the method to identifying and quantifying the unauthorised dimethyl yellow dye in curry was demonstrated.

Keywords: azo dyes; dimethyl yellow; curry; LC-ESI-/MS/MS

Azo type dyes are most frequently studied for analytical food control due to their toxicity risk; the chromophoric azo group, under certain conditions, can be reduced to form confirmed or suspected carcinogenic aromatic amines (AHLSTROM *et al.* 2005). The commercialisation of food contaminated with hot chilli products coloured with azo dyes Sudan I, II, III, and IV led the EU in 2003 to adopt emergency measures (Commission Decision 2003). In April 2009, the Agence Fédérale pour la Sécurité de la Chaîne Alimentaire (AFSCA 2009a) in Belgium recalled curry contaminated with 4-(N,N-dimethylamino)-azobenzene, currently named Dimethyl yellow (C₁₄H₁₅N₃, CAS No. 60-11-7). This dye has been classified as a category 2B carcinogen to humans by the International Agency for Research on Cancer. The Belgium alert was diffused in April 2009, the Rapid Alert System for Food and Feed (RASFF) determined the unauthorised Dimethyl yellow (> 0.50 mg/kg) to be in Madras curry powder from India.

Various analytical techniques and instruments have been employed for the analysis of synthetic co-

lours, such as spectrophotometry (NI & GONG 1997; SAYAR & ÖZDEMİR 1998; BERZAS *et al.* 1999; NI *et al.* 2001, 2009; VIDOTTI *et al.* 2005; PENMAN *et al.* 2006), stripping voltammetry (ALGHAMDI 2005a,b), capillary electrophoresis (SUZUKI *et al.* 1994; LIU *et al.* 1995; MASAR & KANIANSKY 1996; MASAR *et al.* 1996; KUO *et al.* 1998; CHOU *et al.* 2002; DEL GIOVINE & PICCIOLI BOCCA 2003; DOSSI *et al.* 2007), high performance liquid chromatography with UV-VIS, UV-DAD or MS detectors (GREENWAY *et al.* 1992; CHEN *et al.* 1998; GARRIGOS *et al.* 2002 PRADO & GODOY 2002; KIRSCHBAUM *et al.* 2003; CALBIANI *et al.* 2004a,b; GARCIA-FALCON & SIMAL-GANDARA 2005; GIANOTTI *et al.* 2005; ZHANG *et al.* 2005; VIDOTTI *et al.* 2006; KIRSCHBAUM *et al.* 2006; MA *et al.* 2006; ERTAŞ *et al.* 2007; SUN *et al.* 2007; ALVES *et al.* 2008; NOGUEROL-CAL *et al.* 2008; PEREIRA *et al.* 2008; HARTIG *et al.* 2009).

The extraction recovery of various azo-dyes depends strongly on the analysed matrices and for banned azo-dyes is it very important to use analytical methods with very low limit of detection (LOD). In all cases, the LOD by HPLC/MS/MS for azo-dyes re-

sults as lower than those obtained by HPLC/UV-Vis. In addition, MS detection produces more specific structural information. This paper proposes very specific analytical conditions for the rapid detection of dimethyl yellow dye in spices with a LOD lower than the level generally proposed by official organisations for food safety. For example, the document published by AFSCA concerning the protection measures for dimethyl yellow cites the LOD = 15 µg/kg as the analytical value useful to confirm the absence of this illegal dye (AFSCA 2009b).

MATERIAL AND METHODS

Chemicals. HPLC-grade acetonitrile (CH₃CN) was obtained from J.T. Baker (Deventer, the Netherlands). Ethanol and ammonium formate were obtained from Merck (Darmstadt, Germany).

Standards. Dimethyl yellow standard (CAS Number 60-11-7) was obtained from Sigma Aldrich, Milan, Italy (Cod. Prod. 33129). A standard stock solution (680 mg/l) was prepared by dissolving 68 mg of dimethyl yellow standard powder in 100 ml CH₃CN. First dilution (solution A): 1 ml stock solution was diluted to 100 ml with CH₃CN to obtain a 6.80 mg/l solution. Second dilution (solution B): 2 ml solution A was diluted to 10 ml with CH₃CN to obtain a 1.36 mg/l solution.

Spice enrichment with dimethyl yellow. The spice used to verify the response of the adopted extraction method was curry; curry is one of the most complex matrices and potentially offers the most interference in analytical studies. Three

aliquots (2 g each) of the same curry sample, purchased in the market and not containing dimethyl yellow, were enriched with 10 µl, 100 µl, and 250 µl of standard solution B, reaching dimethyl yellow concentrations of 6.8, 68.0, and 170.0 µg/kg, respectively.

Extraction method. The spice (approx. 2 g of enriched curry) was treated with 50 ml CH₃CN in a 75 ml centrifuge tube. The mixture was shaken for 4 h (GFL 3006) and sonicated for 5 min in each hour. The solution was centrifuged for 10 min at 4000 rpm, filtered, and 10 µl of the extract were injected into the LC/MS/MS system.

LC-ESI (+)-MS/MS method. The experiments were carried out with a LC-ESI(+)-MS/MS system using an Agilent 1200 Series HPLC coupled to an Applied Biosystems API 2000 mass spectrometer (Toronto, Canada) equipped with a Turbo IonSpray Source (ESI). **Chromatographic conditions** – A stainless steel column (Macherey-Nagel, Bethlehem, USA) EC/2 Nucleodur 100-5 C18 (150 mm × 2 mm, 110 Å pore diameter, 5.0 µm particle size) was used. Water/5mM ammonium formate (Phase A) and methanol/5mM ammonium formate (Phase B) were used for the mobile phase and the elution gradient was: from 40% to 85% B in 5 min, to 10% B in 5 min, then 100% B for 10 min and return to 40% B in 15 minutes. **ESI (+) MS/MS method** – To establish the appropriate multiple reaction monitoring (MRM) conditions, a test solution was produced by diluting the stock solution 1:1 with mobile phase (1:1 water/methanol, 5mM ammonium formate), to a concentration of 340 µg/l. The test solution was injected by continu-

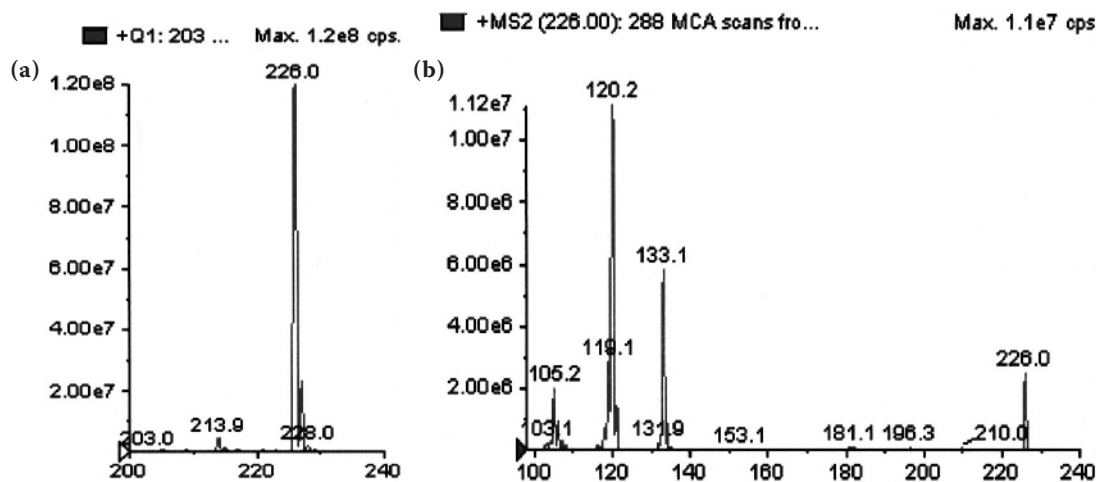
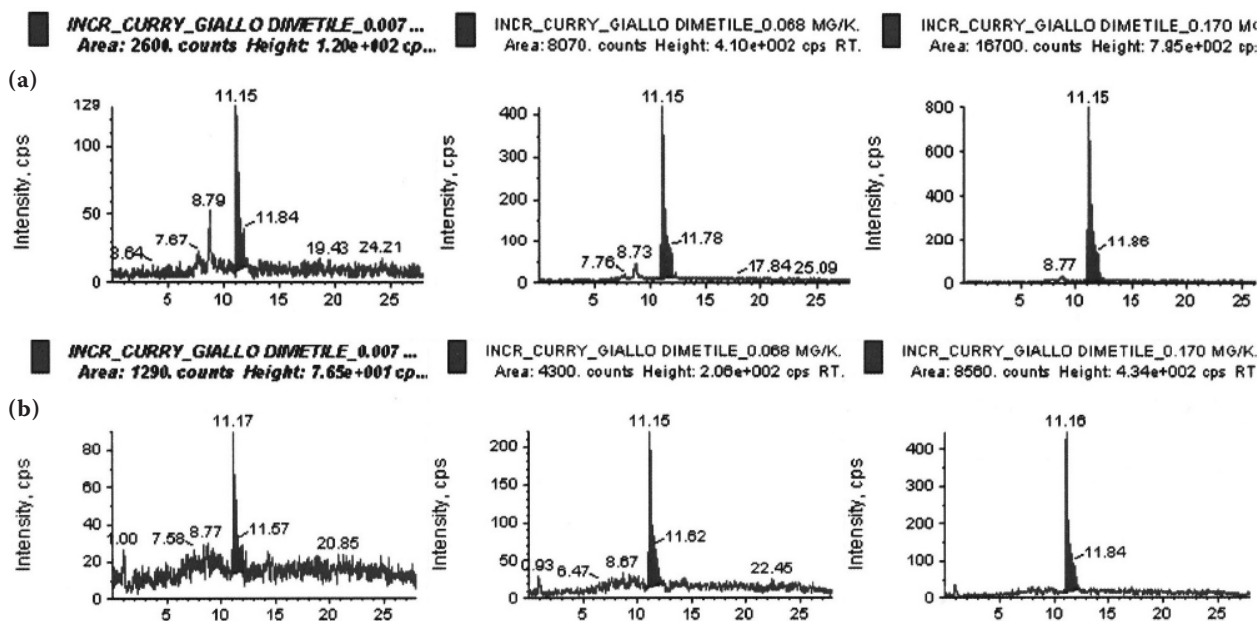


Figure 1. (a) Mass spectrum of dimethyl yellow obtained with a single quadrupole, showing only one chromatographic peak corresponding to ion $[M + H]^+$ $m/z = 226$; (b) daughter ions obtained with multiple reaction monitoring from ion precursor $m/z = 226$ ($m/z = 105.2$ and $m/z = 133.1$)



ous infusion at 5 $\mu\text{l}/\text{min}$ in +Q1 mode and in +MS² mode. The values of DP – Declustering Potential (25 V), FP – Focusing Potential (400 V), EP – Entrance Potential (12 V), CEP – Cell Exit Potential (9 V), CE – Collision Energy (27 V), and CXP – Cell Exit Potential (3 V) were adopted to obtain the 226.0 \rightarrow 105.2 transition. The same values, with the exception of DP (22 V) and CE (45 V), were used to obtain the 226.0 $>$ 133.1 transition.

RESULTS AND DISCUSSION

The mass spectrum of dimethyl yellow, obtained with a single quadrupole, shows only one chromatographic peak corresponding to ion $[M+H]^+$ at $m/z = 226$ (Figure 1a). The daughter ions produced with MRM from ion precursor $m/z = 226$ are shown in Figure 1b ($m/z = 105.2$ and $m/z = 133.1$). Figure 2a shows three peaks, corresponding to daughter ion $m/z = 105.2$, obtained from curry spiked with standard dimethyl yellow at three con-

centrations (6.8, 68.0, and 170.0 $\mu\text{g/kg}$); the peaks corresponding to daughter ion $m/z = 133.1$ appear in Figure 2b. The linear calibration curve shown in Figure 3 was obtained from the areas corresponding to the first transition ($m/z = 105.2$).

When considering the toxicity risk of dimethyl yellow in foods, the quantitative determination is not as important as its identification at a high level of sensitivity. More important, on the contrary, is it to know the LOD and LOQ levels. To determine these values, the calibration curve (Figure 3, $R^2 = 0.9998$) was produced and LOD (5 $\mu\text{g/kg}$) and LOQ (16 $\mu\text{g/kg}$) were calculated from the intercept and slope as reported in Table 1. Concentrations higher than 170.0 $\mu\text{g/kg}$ were not considered for the calibration curve because the quantitative determination of dimethyl yellow is not the aim of this paper for the reason explained before (not tolerance in food).

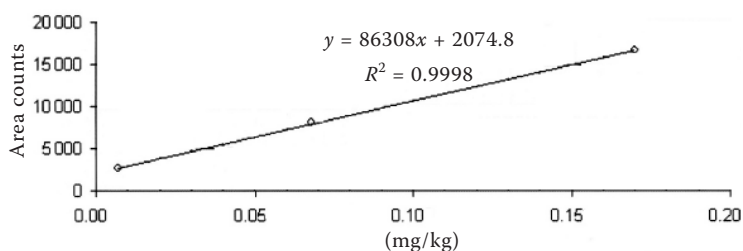


Figure 3. Calibration curve obtained using the area counts corresponding to the first transition (m/z 226 \rightarrow 105.2) at three concentrations (6.8, 68.0, and 170.0 $\mu\text{g/kg}$)

Table 1. Limit of detection (LOD) and limit of quantitation (LOQ) of the LC-ESI-MS/MS method for curry samples calculated from the calibration curve shown in Figure 3

	mg/kg (x)	Area counts (y_i)	$y_t = b \times x + a$	$y_i - y_t$	$(y_i - y_t)^2$	
1	0.007	2 600	2 679	−79	6 238	
2	0.068	8 070	7 944	126	15 930	
3	0.170	16 700	16 747	−47	2 231	
		$a = 2\,074.8$	$b = 86308.3$			
	$S(y_i - y_t)^2$	$S_{y/x}$	LOD (mg/kg)	LOQ (mg/kg)	R	R^2
	24 399	156	0.005	0.016	0.9999	0.9998

$S_{y/x} = \sqrt{[(\sum(y_i - y_t)^2)/(n - 2)]}$; LOD = $3 \times S_{y/x}/b$ limit of detection; LOQ = $9 \times S_{y/x}/b$ limit of quantification

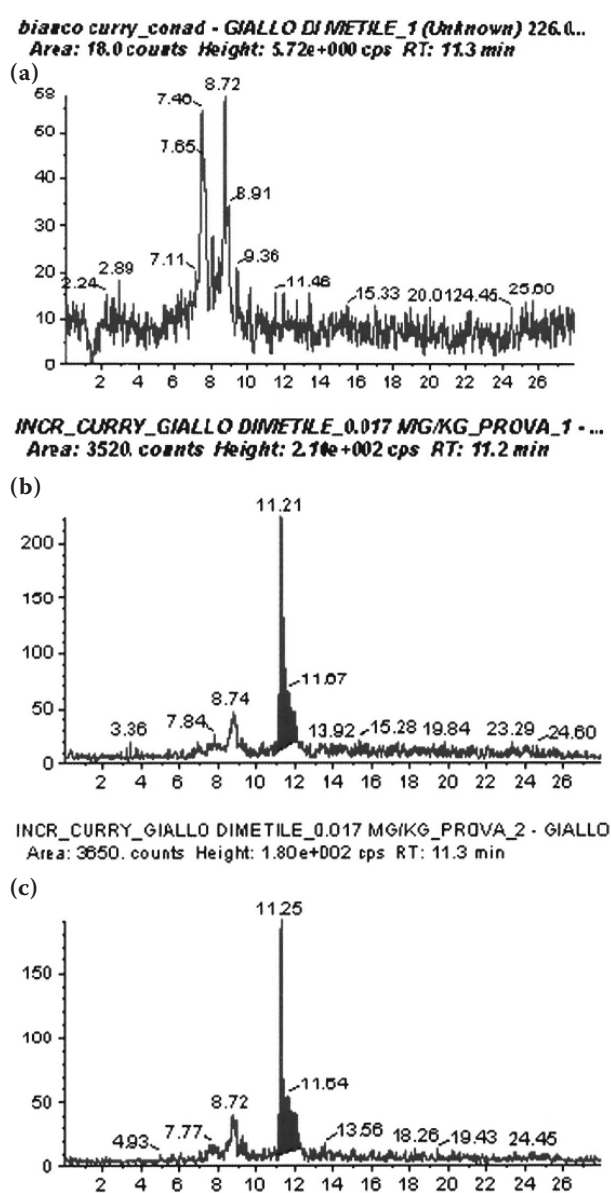


Figure 4. LC-ESI-MS/MS traces obtained from: (a) curry powder (blank matrix), (b) and (c) duplicate curry powder samples spiked with 17.0 µg/kg dimethyl yellow

No. 5 (Nordic Committee on Food Analysis 1997). The suggested method was applied to duplicate analyses of dimethyl yellow in each sample and was applied to 10 different curry samples enriched with different amounts of dimethyl yellow, measured on different days. The RSDr value for 10 samples of curry was 2.7%.

Considering the calibration curve was produced from the data obtained by matrix enrichment, the recovery corrections are included in the method. To verify the high recovery also at lower concentrations of dimethyl yellow near the LOQ value, two identical additions were made to the curry sample not containing dimethyl yellow: 25 µl of standard solution B was added to 2 g of matrix, resulting in a 17 µg/kg concentration of dimethyl yellow. Figure 4 shows three chromatograms, corresponding to the blank matrix (Figure 4a) and the duplicate samples of the enriched matrix (Figures 4b and c, with area values of 3520 and 3650). The recovery values correspond to 100.0% and 105.8%, respectively, and the efficiency in the identification of dimethyl yellow at the concentration levels of the LOQ order were confirmed.

CONCLUSION

The LC-ESI(+)-MS/MS fast method proposed seems to be adequate to detect the banned dimethyl yellow azo-dye since the detection limits are lower than obtained with other methods. The extraction method adopted for curry, the matrix showing more interferences than other spices, produced high recovery and good repeatability data; moreover, the LOD and LOQ values proved to provide unambiguous identification as requested for food matrices where the presence of the specific azo

dye is banned. The experiences were developed in particular to ameliorate the data quality in correspondence with LOD and LOQ levels, the quantitative determination at higher levels being not useful for food safety control.

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