GC/MS-MID Determination of Safrole in Soft Drinks

Monica Bononi, Alessandro Fossati, Elisabetta Lubian, Fernando Tateo[1] and Sergio Fasan[2]

[1]Laboratorio Analisi Alimenti "HRGC/MS/HPLC", Di.Pro.Ve., Faculty of Agriculture, University of Milan, Italy
[2]CHELAB Laboratories, Resana (Treviso), Italy

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Introduction

Safrole [5-(2-propenyl)-1,3-benzodioxole] is a compound belonging to the class of alkenyl benzenes. It is classified as "H/M" (high/medium) in the Nettox priority list (1) as far as myristicin [4-methoxy-6-(2-propenyl)-1,3-benzodioxole], apiole [4,7-dimethoxy-5-(2-propenyl)-1,3-benzodioxole], eugenol [2-methoxy-4-(2-propenyl)-phenol], methyl eugenol [1,2-dimethoxy-4-(2-propenyl)-benzene], elemicin [1,2,3-trimethoxy-5-(2-propenyl)-benzene], estragole [1-methoxy-4-(2-propenyl)-benzene] and other natural substances having chemical structures to be considered related to possible toxicological effects.

Safrole content in non alcoholic beverages is limited to 1 mg/kg by CE Directives N. 88/388 and 91/71 concerning "flavourings for use in foodstuffs and source materials for their production". The possibility to detect concentrations lower than 0.1 mg/l has a great impact on the recognition of convenience goods for which the lower content of safrole constitutes a quality parameter for safety reason.

The analytical methods available for a GC-based quantitative determination of safrole (2–4) suggest non-standardisable extraction procedures by solvent or/and steam distillation. These operations are critical points in relation to recovery values, repeatability and detection limits. Moreover, no validation data are reported for these methods. Our experience shows RSDs values in all cases higher than 8.0 % at concentrations lower than 100.0 µg/l.

Zubillaga and Maerker (5) suggest the use of a "dry column" procedure followed by TLC or HPLC isolation before GC analysis. Nevertheless this method is applicable only at safrole levels of 100 mg/kg.

The present communication reports an improved analytical method considering the standardisation of the extraction procedures, allowing the evaluation of very low safrole concentrations. The method foresees a preliminary extraction by hydrodistillation using a Clevenger-type apparatus, followed by GC/MS-MID (Multiple ion detection) analysis.

Furthermore we verify the applicability of that method on a commercial soft drink beverage ("cola" type).

Materials and methods

Standard and reagents

The following standards were used for our analytical purpose:

A Safrole (Aldrich, Milan, Italy) standard solutions at concentration between 500 and 50 mg/l in methanol: aliquots ranging between 25.0 and 2.5 mg of safrole, accurately weighed, are dissolved in 50 ml with methanol.

B Camphor (Aldrich, Milan, Italy) standard reference solution at 500 mg/l: about 25.0 mg of camphor, accurately weighed, are dissolved 50 ml with methanol.

C Limonene standard: Aldrich (Milan, Italy)

Identification of the linear concentration range

Several 500 ml volumes of an aqueous solution simulating the beverage (i.e. containing the same amount of sugars and organic acids) were placed in a Clevenger-type apparatus: to each of them 0.1 ml of safrole solutions at concentrations ranging of 500 to 50 mg/l (A in "Standard and reagents"), 0.1 ml of camphor standard solution (B in "Standard and reagents"), 1 ml of limonene (stripping agent and recovery solvent for safrole) and about 20 g of sodium chloride were added. These solutions contained 100.0 µg/l of camphor and safrole between 10.0–100.0 µg/l . These solutions were hydrodistilled in a Clevenger-type apparatus for two hours and the organic fractions collected were analysed by GC/MS-MID. The graph was constructed reporting in abscissa the ratio between safrole peak area (AS) and camphor peak area (AC), both expressed in "area counts". In ordinate safrole standard solutions concentrations (µg/l) are reported.

The volume of limonene recovered, not lower than 0.85–0.90 ml, does not influence the quantitative data obtained for safrole because the calibration graph depends on the area ratio safrole/camphor. Moreover, it's been verified that each safrole/camphor ratio in limonene was strictly reproducible in the overall range of linearity (average RSDs lower than 0.1%). Evidently that depends on the high volatility of both safrole and camphor, which gives high comparable recoveries.

Soft drink extraction

A 500 ml volume of the soft drink was placed in a Clevenger-type apparatus together with 1 ml of limonene, about 20 g of sodium chloride, 0.1 ml of camphor

standard solution (B in "Standard and reagents") and 0.1 ml of methanol. The mixture was extracted for two hours and the organic fraction collected was analysed by GC/MS-MID.

Recovery experiments

Recovery experiments were performed in triplicate by spiking the soft drink samples with safrole at levels between 30.0–80.0 µg/l.

GC/MS-MID analysis conditions

Analyses were performed on a Shimadzu mass spectrometer QP 5050A equipped with a gas chromatograph Shimadzu GC-17A; GC conditions were: an SPB™-5 fused-silica capillary column, 30 m × 0.25 mm i.d., 0.20 µm film thickness (Supelco, Milan, Italy); column temperature, 60° C (1 min) to 230° C (20 min) at 2° C/min; injector temperature, 220° C; injection mode, split; split ratio 1/20; injection volume 0.2 µl; carrier gas (He) at 1 ml/min.

MS conditions were: GC/MS interface temperature, 250° C; acquisition parameters, MID. Selected ions were m/z 95–108–152 for the internal standard (camphor) and m/z 161–162–163 for safrole.

Results and discussion

Table 1 reported the internal validation data for the proposed method: the recovery values ranged between 80–93 % (RSDs: 2.7–5.1 %). The limit of detection (LOD) was 10.0 µg/l based on a conventional signal/noise ratio of 3:1. The limit of quantification (LOQ) was 30.0 µg/l.

Table 1
Internal validation data for the proposed GC/MS method for quantitative determination of safrole in "cola type" beverages

Limit of detection (LOD) (mg/l)	10.0[a]
Limit of quantification (LOQ) (mg/l)	30.0
Recovery	80–93 % (RSDs = 2.7–5.1 %)
Linear concentration range (mg/l)	10.0–100.0 (Y = 111.7X, R^2 = 0.9985)

[a] Calculated as three times the baseline noise.

In figure 1 the linear concentration range for the method is reported: under the operating conditions described, safrole response was linear over a concentration range of 10.0–100.0 µg/l (Y = 111.7X, R^2 = 0.9985).

Moreover the method was applied to a commercial "cola" beverage giving a result lower than LOQ.

In conclusion the recoveries obtained with spiked samples and the other validation data give evidence of the accuracy and applicability of the proposed method: it

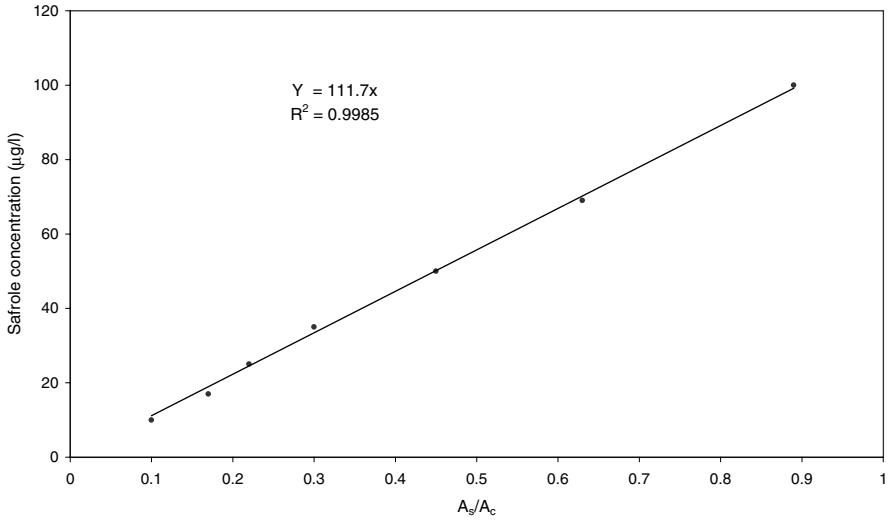

Figure 1 **Calibration curve for safrole quantitative determination over a linear concentration range of 10.0–100.0 µg/l obtained under the operating conditions described in the "Materials and methods" section**
A_S = safrole peak area
A_C = camphor (internal standard) peak area

is shown to be rapid, suitable to detect low concentration values and simpler than the current GC methods mentioned in bibliography.

These considerations suggest the use of that method as suitable for routine analysis on foodstuffs.

Summary

Safrole [1,3-benzodioxole, 5-(2-propenyl)-] is a limited substance according to the CE Directives N. 88/388 and 91/71 concerning "flavourings for use in foodstuffs and to source materials for their production". At today the GC analytical methods proposed for the quantitative determination of safrole are critical as far as concerning reproducibility, recovery values and detection limits. This note describes an improved analytical method to quantify safrole in "soft" drinks. Average recoveries of safrole from samples spiked at levels from 30.0 to 80.0 µg/l ranged from 80 to 93 % with good reproducibility (RSD = 2.7 at 30.0 µg/l). Limits of detection (LOD) and quantification (LOQ) were 10.0 µg/l and 30.0 µg/l respectively. The proposed method is also suitable for routine analysis.

Zusammenfassung

Safrol (1,3-Benzodioxol, 5-[2-Propenyl]-) ist eine Substanz gemäss EWG-Richtlinien 88/388 und 91/71 über «Aromen zur Verwendung in Lebensmitteln und Ausgangstoffe für ihre Herstellung». Bis heute sind die zur quantitativen Bestimmung von Safrol vorgeschlagenen GC Analysemethoden hinsichtlich Wiederholbarkeit, Wiederfindung und Nachweisgrenzen kritisch. Der vorliegende Bericht beschreibt eine verbesserte Analysenmethode zur quantitativen Bestimmung von Safrol in alkoholfreien Getränken. Die mittlere Wiederfindung von Safrol bei Proben von 30,0–80,0 mg/l liegt zwischen 80 und 93 % bei einer guten Wiederholbarkeit (RSD = 2,7 bei 30,0 mg/l). Die Nachweisgrenze (LOD) und der mengenmässige Nachweis (LOQ) betragen 10,0 mg/l bzw. 30,0 mg/l. Die vorgeschlagene Methode ist auch für Routineanalysen geeignet.

Résumé

Le safrole (1,3-benzodioxol, 5-[2-propenyl]-) est une substance régie par les Directives CE n. 88/388 et 91/71 concernant les «arômes à utiliser pour les denrées alimentaires et les matières premières pour leur production». Les méthodes d'analyse par chromatographie gazeuse proposées à ce jour pour la determination quantitative du safrole posent des problèmes aux niveaux de la reproductibilité, des taux de récupération et des limites de détection. Cet article décrit une méthode analytique améliorée pour quantifier le safrole contenu dans les boissons non alcoolisées. La moyenne de récupération du safrole sur la base d'échantillons dopés, à des concentrations comprises entre 30,0 et 80,0 mg/l allait de 80 à 93 % avec une bonne reproducibilité (RSD = 2,7 à 30,0 mg/l). Les limites de détection (LOD) et de quantification (LOQ) étaient de 10,0 mg/l et de 30,0 mg/l respectivement. La méthode proposée convient également pour les analyses de routine.

Key words: Safrole, Soft drinks, GC/MS determination

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Corresponding author: Prof. Fernando Tateo, Laboratorio Analisi Alimenti "HRGC/MS/HPLC", Di.Pro.Ve., Faculty of Agriculture, University of Milan, Via Celoria 2, I-20133, Milano, E-mail: fernando.tateo@unimi.it