



# Modified QuEChERS Approach for Analysis of Synthetic Food Dyes in Jellies and Smarties

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Received: 19 October 2017 / Accepted: 11 December 2017 / Published online: 12 January 2018  
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## Abstract

This work deals with the application of the QuEChERS method for extraction of synthetic food dyes (SFD) from confectionery samples: tartrazine (E102), quinoline yellow (E104), cochineal red A (E124), allura red (E129), patent blue V (E131), and brilliant blue (E133). Extraction conditions, like extraction solvent and its amount, sample amount, the pH adjustment and addition of salt, were optimised. The 5 mL of sample, 2 mL of propan-1-ol as extraction solvent with addition of 0.6 g NaCl, 2.4 g MgSO<sub>4</sub>, and 0.75 mL 35% HCl were experimentally found to be optimal. Analyses of extracts were performed by liquid chromatography with detection of SFD in the visible region. Recoveries of SFD were in the range of 79.3–100.0% with intra-day precision below 4.6% RSD. The modified QuEChERS method connected with liquid chromatography has been applied to quantify SFD in samples of confectionery (smarties and jellies). The whole procedure was evaluated as a fast method for screening of SFD content in samples of jellies and smarties.

**Keywords** QuEChERS · Extraction · Synthetic food dyes · HPLC-Vis · Confectionery

## Introduction

Food dyes are substances giving to foodstuffs a colour which would not be sufficient without their use or reconstruct a colour that has been damaged or weakened during the technological process. Currently, the food industry considers colour as an important criterion for food choice, thus synthetic colourants are frequently used to improve the aesthetic quality (Chequer et al. 2012), and in some cases are used to mask defective food when less desirable food seem more desirable. Their use range and dosage are restricted strictly in the world. Many kinds of synthetic dyes were admitted to be used as food colourant (Sun et al. 2013).

It was published study that examined the effect of some dyes (including E102, E124 and E129 whose was tested in our work)

and sodium benzoate on children's behaviour (McCann et al. 2007). This study concluded that synthetic dyes or chemical preservative sodium benzoate in the diet of children may cause increased hyperactivity. This work followed previous studies that also pointed to a possible connection between behavioural disorders at children in the context of some synthetic dyes (Bateman et al. 2004). However, some other studies have contested their results and indicated on the lacks, e.g. confusion hyperactivity diagnosed with ADHD (attention deficit hyperactivity disorder) or possible influence on results by parents who have observed changes in the behaviour of children (Eigenmann and Haenggeli 2007).

As part of the safety evaluation of food dyes risk assessors like EFSA (European Food Safety Authority) establish, when possible (i.e. when sufficient information is available), an acceptable daily intake (ADI) for each substance. The ADI is generally derived by looking at the highest intake level at which substances do not cause harmful effects in animal experiments and applying a safety factor (typically of 100) to account for differences between humans and animals (EFSA 2014).

The presence of food additives (including dyes) in food-stuff must be marked on the packaging either by the name or by code number. Numeric E code is the designation used to identify substance within the European Union. The allocation

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**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s12161-017-1130-9>) contains supplementary material, which is available to authorized users.

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of this code means that the additive has passed a safety assessment and was allowed to use in the European Union. Substances authorised in the European Union are listed in regulation of the European Parliament and Council No 1129/2011, Annex II (Commission 2011).

Traditional method for the extraction of food dyes from foodstuffs utilises pure, unbleached wool (Nollet and Toldrá 2015). It is based on extraction of dyes by white defatted wool fibre. Acetic acid solution of the sample is boiled with white wool (first purified by boiling in dilute sodium hydroxide, then in water). After discoloration of solution, the wool is washed with cold water (elimination of natural dyes), and then the dyes are stripped from it by boiling the wool with dilute ammonia. But this method has several disadvantages, like changes in colours of dyes due to their thermal and pH instability, and low effectivity in samples containing large amounts of proteins, fats and saccharides which compete with dyes in binding on wool fibres (Gonzalez et al. 2002; Nollet 2000).

Many new extraction methods were published for isolation of synthetic food dyes (SFD). These are based on cloud point extraction (El-Shahawi et al. 2013), solid-phase extraction (Rejczak and Tuzimski 2017; Siangproh et al. 2013; Soyлак and Cihan 2013), matrix solid-phase dispersion (Rejczak and Tuzimski 2017), accelerated solvent extraction (Liao et al. 2012) or microwave-assisted extraction (Sun et al. 2013). QuEChERS (quick, easy, cheap, effective, rugged, and safe) procedure seems to be a perspective method for food dyes isolation. It is the extraction method used primarily for the analysis of a wide range of pesticide residues in fruits, vegetables, grains and products made from them. This technique involves a few simple steps leading to the transfer of the analytes into the extraction solvent (acetonitrile is used for pesticides). The extract could be used for further analysis (Lehotay et al. 2010; Wiłkowska and Biziuk 2011). This method is mostly used primarily for mentioned extraction of pesticide residues in food samples, but there are pre-conditions for the use of extraction of other analytes from different samples, like routine contaminant control of food products, feedstuff and environmental samples (Rejczak and Tuzimski 2015).

The aim of this study is application of the QuEChERS method's principles for extraction of SFD from foodstuffs, especially from confectionery samples. It could lead to finding a fast, easy and effective method as an alternative to still commonly used method based on the extraction of dyes using wool fibres. Although producers of confectioneries often use natural dyes too, the fast, easy and effective method for analysis of SFD is need for quality control of those products. Extracts obtained by proposed method could be easily measured by spectrophotometric method in the visible region at appropriate wavelengths of individual dye. SFD in extracts can be also determined by high-performance liquid chromatography (Bonan et al. 2013; Zou et al. 2013). For this study, six SFD were selected as markers according to their common appearance in confectionery samples.

## Materials and Methods

### Reagents

Standards of synthetic dyes tartrazine (E102, 99%), quinoline yellow (E104, 97%), cochineal red A (E124, 99%), allura red (E129, 98%), patent blue V (E131, 97%) and brilliant blue (E133, 97%) were purchased from Fluka Analytical (Steinheim, Germany). Stock standard solutions were prepared by dissolution of appropriate amount of standard (see Table S1, supplementary material) in 25 mL of deionised water. Working solutions for extractions were prepared by 50 times dilution of stock solutions in deionised water to have absorbance equal to 1.0 by spectrophotometric analysis in a 1-cm cuvette.

Acetonitrile, sodium acetate, methanol, ethanol, propan-1-ol, propan-2-ol, NaCl, MgSO<sub>4</sub> and 35% HCl, all with purity for analysis or better, were purchased from Sigma-Aldrich (Steinheim, Germany). Water was purified using a Milli-Q® water purification system (Millipore SAS, Molsheim, France).

### Samples

Confectionery samples (smarties and jellies) were purchased in local supermarkets. Jellies (approx. 3 g) were dissolved in a small amount of distilled water at 70 °C, and, after cooling, transferred to 25-mL volumetric flasks and filled up to the mark with deionised water. From samples of smarties (approx. 1 g), a layer of dyes was washed away with deionised water (to discoloration of the sample) into a 10-mL volumetric flask and filled up to the mark with deionised water.

## Instrumentation and Methods

### Modified QuEChERS Extraction

Extraction was performed in a plastic closable 25-mL test tube (Supelco, Bellefonte, PA, USA). Five milliliters of an aqueous solution of synthetic dyes and 2 mL of propan-1-ol was pipetted into a test tube. Then, 0.6 g of NaCl, 2.4 g MgSO<sub>4</sub> and 0.5 mL 35% HCl were added, and the tube was closed and shaken vigorously to dissolve of added salts. Two separate phases were formed in a test tube; the upper organic phase was analysed without any pretreatment.

### Spectrophotometry

The HELIOS Gamma UV-Vis spectrometer by Thermo Fisher Scientific (Waltham, MA, USA) was employed for all spectrophotometry analyses. Spectra were measured in a cell with a 1-cm optical path against a propane-1-ol in the range of 400–800 nm. Absorption maxima of appropriate wavelength were evaluated in obtained spectra.

**Table 1** Gradient elution program for separation of synthetic dyes mixture; A acetonitrile; B water with sodium acetate (10 mM) modified by HCl to pH 6.0

Time (min)	Flow rate (mL/min)	Mobile phase	
		A (%)	B (%)
0	0.5	0	100
5	0.5	0	100
10	0.5	5	95
10.5	0.75	5	95
15	0.75	10	90
15.5	1	10	90
25	1	20	80
40	1	100	0

### Chromatographic System

The obtained extracts were analysed by a HPLC coupled with Vis detector (HPLC-Vis) using a chromatographic system consisting of high-pressure pump LC1150, automatic dosing device LC1650, dual channel UV-Vis detector LC1210 (all from GBC, Regents Park, Australia). The system was also equipped with a degasser ERC-3415 (ERC, Tokyo, Japan).

The amount of extract for analysis was 20  $\mu$ L. The separation was carried out using LiChrospher® 100 CN (5  $\mu$ m) 250  $\times$  4 mm column with LiChrospher® 100 RP-18e (5  $\mu$ m) 4  $\times$  4 mm guard column in LiChroCart® 250-4 HPLC-cartridge implementation, all from Merck (Darmstadt, Germany). The mobile phase A was acetonitrile. The mobile phase B was water with sodium acetate (10 mM) modified by HCl to pH 6.0. The gradient elution was based on (Kirschbaum et al. 2003) and slightly modified. Final conditions are shown in Table 1. Detection was carried out at 420 (E102, E104), 510 (E124, E129) and 630 nm (E131, E133). The individual compounds were identified by the comparison of retention times with retention times of standards, and the identification was verified by the method of standard addition. The content of individual compounds was determined by the standard addition and calibration curve methods.

### Design of Experiment

An orthogonal central composite design was performed by varying the salts addition (NaCl and MgSO<sub>4</sub> 1:4 m/m) from 1 to 3 g (coded as  $x_1$ ), the pH modified by 35% HCl addition from 0 to 1 mL (coded as  $x_2$ ), and the extraction solvent volume from 2 to 5 mL (coded as  $x_3$ ). Extracts were analysed by chromatography (for conditions see ‘[Chromatographic System](#)’). The data obtained from analytical method were treated using the software Statistica CZ, version 12 (Dell software, Prague, Czech Republic). Statistical models, constructed using second-order modelling, were created for each tested synthetic dye. The whole design consisted of 20 experimental points, which have been used to estimate effects of each factor on the extraction efficiency for individual dyes. Individual conditions of all experimental points together with observed and predicted values are summarised in the supplementary section (Table S2, supplementary material).

## Results and Discussion

### Chromatographic Method

HPLC-Vis analyses were performed according to the conditions given in ‘[Chromatographic System](#).’ The retention times of standards of individual dyes are presented in Table 2. Additionally, individual compounds were identified also by standard addition method.

For the calculation of the limit of quantification—LOQ and the limit of detection—LOD were used the same approach as in our previous publication (Bajerova et al. 2014). The LOQ were obtained by dilution method, where individual standard solutions were diluted to obtain signal equal to  $10 \cdot s_0$ , where  $s_0$  is noise at the point of determination, LOD were calculated from relationship  $LOD = 3/10 \text{ LOQ}$ ; the values found are shown in Table 2.

Based on optimised conditions of QuEChERS procedure (see ‘[Modified QuEChERS Extraction](#)’), five point calibration curves were measured and constructed for the determination

**Table 2** Retention time and calibration characteristics of tested dyes,  $n = 3$ 

Dye	$T_R$ (min)	LOD ( $\mu$ g/mL)	LOQ ( $\mu$ g/mL)	Calibration range ( $\mu$ g/mL)	Calibration equation	$R^2$
E102	5.3	0.14	0.45	LOQ—18.43	$y = 82,106x + 125,242$	0.9971
E104	13.9	1.45	4.83	LOQ—22.18	$y = 24,950x - 28,259$	0.9962
E124	12.6	0.10	0.34	LOQ—38.11	$y = 52,321x - 26,041$	0.9959
E129	17.1	0.06	0.18	LOQ—32.06	$y = 57,534x - 58,743$	0.9998
E131	29.0	0.35	1.15	LOQ—8.56	$y = 25,056x + 5736$	0.9927
E133	24.1	0.16	0.52	LOQ—2.94	$y = 136,452x - 27,090$	0.9941

of individual dyes. Calibration ranges were selected according to expected amounts of individual dyes in real samples. The equations of linear regression and the respective coefficients of determination ( $R^2$ ) are shown in Table 2.

## Extraction Method Optimization

### Spectrophotometry—Choice of Solvent

Extraction was performed at the conditions found during the optimization studies with the standards of dyes. Original method QuEChERS applied on pesticide residues uses acetonitrile as an extraction solvent. But acetonitrile was not suitable for extraction of SFD, because SFD (except E131) did not pass to the acetonitrile phase. Therefore, homologous series of alcohols was tested. Methanol was not suitable because no phase separation during extraction was observed. Ethanol was not suitable as well because of significant dilution of organic phase by water. Propan-1-ol and propan-2-ol were evaluated as the suitable extraction solvents. Moreover,

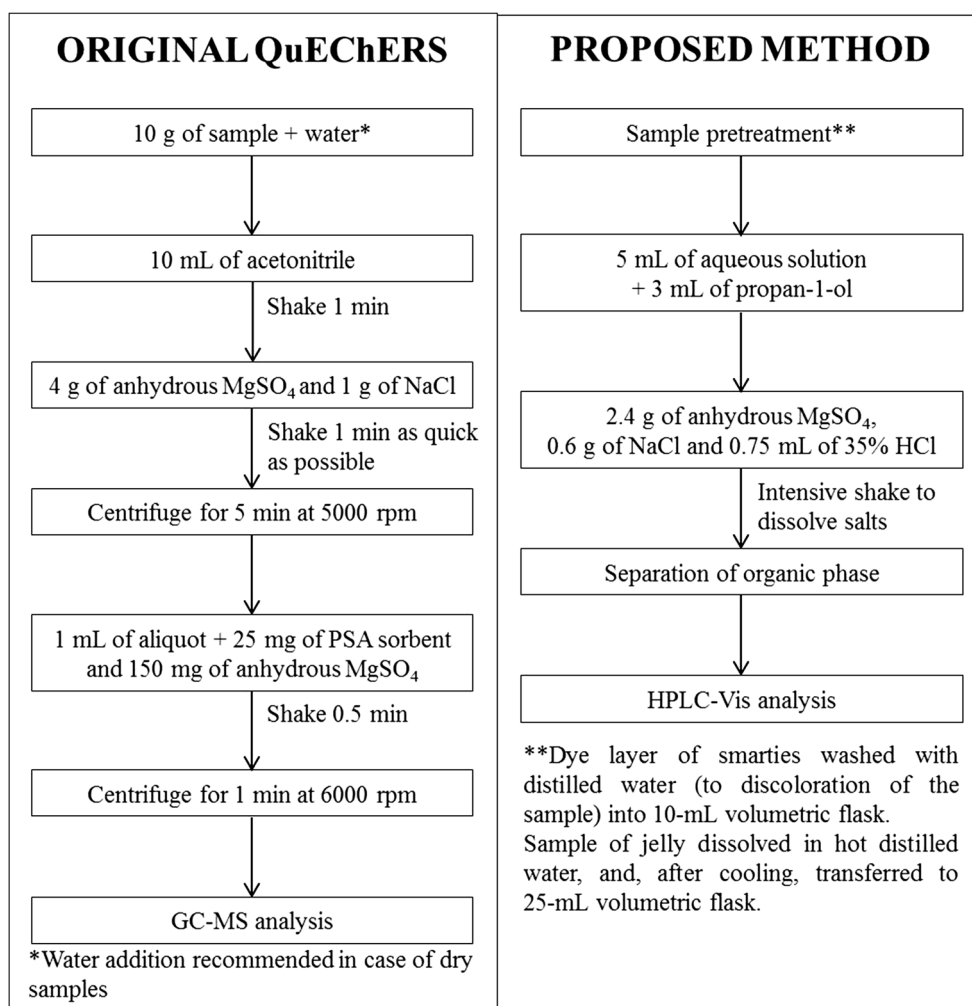
propan-1-ol gave higher yield of extraction after addition of 35% HCl.

Original method QuEChERS for extraction of pesticides applied primary-secondary amine (PSA) sorbent to remove of fatty acids. In this case, this step was not necessary. The reasons are based on the types of analysed samples. In the jelly no fatty acids are contained, and in the case of smarties only dye layer was washed. When saccharides are taken into an account, their solubility in propan-1-ol is significantly lower than in water. Moreover, it was confirmed by analysis of real samples that application of PSA (to remove fatty acids) or C18 (to remove saccharides) sorbents was not necessary.

### Statistical Evaluation of Extraction

Significant parameters at  $P$  value 0.05 from statistical evaluation of chromatographic data are shown in supplementary section (see Table S3, supplementary material) together with the square roots of the determination coefficient ( $R^2$ ) of the models with linear terms, quadratic terms and interaction

**Fig. 1** Comparison of the proposed method with the original QuEChERS (Anastassiades et al. 2003) adapted to confectionery samples



**Table 3** Extraction recovery and enrichment factor of all tested dyes

Target compound	Enrichment factor	Recovery (%)	Precision (% RSD)	
			Intra-day ( <i>n</i> = 5)	Inter-day ( <i>n</i> = 5)
E102	2.33	99.5 ± 4.6	4.6	11.6
E104	1.41	83.1 ± 3.6	4.3	2.8
E124	2.20	84.9 ± 2.1	2.5	8.3
E129	2.38	79.3 ± 3.6	4.6	4.7
E131	0.98	100.0 ± 1.8	1.8	4.9
E133	2.26	87.6 ± 0.8	0.9	4.3

*n* number of extraction process replications

**Table 4** Comparison of recoveries of SFD for various method of sample treatment and proposed method

Dye	Method*	Recovery (%)	Sample	References
E102	QuEChERS	99.5	Confectionery	This study
	ASE	78.3–84.9	Grilled meat	Liao, Li, & Luo, 2012
	Dilution	90.4–108.7	Soft drinks	Li, Zhou, Tong, & Jia, 2013
	Dilution	87.6	Beverages	Bonan, Fedrizzi, Menotta, & Elisabetta, 2013
E104	QuEChERS	83.1	Confectionery	this study
	Dilution	75.7	Beverages	Bonan, Fedrizzi, Menotta, & Elisabetta, 2013
E124	QuEChERS	84.9	Confectionery	this study
	ASE	76.9–83.6	Grilled meat	Liao, Li, & Luo, 2012
	S-L	83–91	Feed	Zou, He, Yasen, & Li, 2013
	S-L	87–92	Poultry meat	Zou, He, Yasen, & Li, 2013
	S-L + SPE	75.0	Solid food matrices	Bonan, Fedrizzi, Menotta, & Elisabetta, 2013
	Dilution	101.1	Beverages	Bonan, Fedrizzi, Menotta, & Elisabetta, 2013
E129	QuEChERS	79.3	Confectionery	this study
	ASE	80.9–84.6	Grilled meat	Liao, Li, & Luo, 2012
	S-L	90–94	Feed	Zou, He, Yasen, & Li, 2013
	S-L	85–97	Poultry meat	Zou, He, Yasen, & Li, 2013
	Dilution	93.5–107.9	Soft drinks	Li, Zhou, Tong, & Jia, 2013
	S-L + SPE	90.7	Solid food matrices	Bonan, Fedrizzi, Menotta, & Elisabetta, 2013
	Dilution	86.1	Beverages	Bonan, Fedrizzi, Menotta, & Elisabetta, 2013
E131	QuEChERS	100.0	Confectionery	this study
	USE	101.8	Mint-flavoured sweets	Minioti, Sakellariou, & Thomaidis, 2007
	Dilution	83.2	Beverages	Bonan, Fedrizzi, Menotta, & Elisabetta, 2013
E133	QuEChERS	87.6	Confectionery	this study
	ASE	83.3–84.7	Grilled meat	Liao, Li, & Luo, 2012
	Dilution	66.3	Beverages	Bonan, Fedrizzi, Menotta, & Elisabetta, 2013

\*S-L solid-liquid extraction, SPE solid-phase extraction, USE ultrasound assisted extraction, ASE accelerated solvent extraction



between linear terms which ranged from 0.8697 to 0.9832.  $R^2$  considering only linear effects in the models were in range 0.1473–0.8750, and increases to the range 0.7436–0.9014 with introduction of quadratic terms. These results confirmed that also evaluation of interactions between linear terms is necessary. However, optimal conditions varied for individual dyes, especially in the term of HCl addition. Therefore, the evaluation of one global model was performed.

The optimised conditions for extraction of all tested dyes in one extraction procedure (see ‘[Modified QuEChERS Extraction](#)’) were determined by evaluation of three-dimensional response surfaces (Fig. S1, supplementary material). These are the graphical representations of the global model. Each of graphs represents dependency of the response (expressed as desirability) on independent variables. The effects of parameters on the peak areas and interaction between them can be estimated from the biaxial contour plot in the base of three-dimensional plot and from the shape of three-dimensional surface. Dark colour indicates increase of peak area and surroundings of optimum conditions.

A comparison between the original QuEChERS approach and the modifications proposed in this paper is summarised in Fig. 1.

## Recovery and Enrichment Factor

Recovery and enrichment factor were determined by evaluation of responses of dyes by HPLC-Vis. For calculation of recovery, the handmade samples of jelly with spiked amount of dyes were used, and the amount of dye determined after extraction was related to the amount of dye presented in sample of jelly.

Enrichment factor was calculated as a ratio of concentrations of standard dye solution before and after extraction. Results are shown in Table 3 together with values of both intra-day (five replications in 1 day) and inter-day precisions

(results obtained in five consecutive days). Samples for determination of extraction recovery and enrichment factor were prepared in the form of jelly. Concentrations of individual SFD were three times the concentration of LOQ. Recoveries ranged from 79.3 (E129) to 100.0% (E131), and enrichment factors were in the range of 0.98–2.38 (depending on the type of dye).

Comparison of SFD recoveries obtained by various methods and proposed approach is shown in Table 4. Recoveries of SFD using proposed method are comparable or better (except E129) than recoveries published by other authors.

## Analysis of Real Samples

Samples of smarties and jellies were pretreated according to procedure shown in ‘[Samples](#).’ Modified QuEChERS was used for determination of SFD in all tested samples (see ‘[Modified QuEChERS Extraction](#)’). Obtained extracts were analysed by method HPLC-Vis (for chromatographic conditions see ‘[Chromatographic System](#)’), and results and comparison to conventional wool dyeing technique are summarised in Table 5. All experiments were performed in triplicate. Results obtained by modified QuEChERS method ranged from 94.7 to 112.6% compared to conventional method. Found amounts of individual dyes in all tested samples were much lower than appropriate ADI levels. Highest content was observed in red smarties (318.02  $\mu\text{g/g}$ ).

## Conclusion

It was determined a new extraction approach for isolation of synthetic food dyes from confectionery samples. This procedure is based on QuEChERS method. In comparison to the common method based on the extraction of SFD on wool

**Table 5** Comparison of results obtained by proposed QuEChERS-HPLC-Vis method and conventional method based on the wool fibre extraction

Sample	Identified dyes	Proposed		Conventional	
		Dye amount ( $\mu\text{g/g}$ )	RSD (%)*	Dye amount ( $\mu\text{g/g}$ )	RSD (%)**
Jelly bears red	E124	80.54	7.56	84.05	6.69
Jelly bears yellow	E104	112.98	7.93	119.07	8.57
Jelly bears green	E104	67.01	5.21	69.66	3.63
	E131	20.32	9.30	20.82	10.20
Red smarties	E129	318.02	1.78	298.80	6.08
Blue smarties	E133	23.71	0.55	25.05	0.83
Yellow smarties	E102	141.72	5.45	146.00	3.62
Green smarties	E102	120.82	2.95	119.60	5.32
	E133	9.80	3.60	8.70	7.61

\*Relative standard deviation for QuEChERS-HPLC/VIS method ( $n = 3$ )

\*\*Relative standard deviation for wool fibre extraction-HPLC/VIS method ( $n = 3$ )

fibre, the proposed modified QuEChERS method reduces solvent consumptions and time required, provides comparable results with suitable recovery and meets the principles of QuEChERS. It means that method is quick, easy, cheap, effective, rugged and safe. Additionally, the proposed method is in a good agreement with the principles of green analytical chemistry, especially in the case of solvent and time consumptions. The biggest benefit of the presented method is its capability for fast synthetic dyes content screening in laboratories. The extraction method is feasible with basic laboratory equipment without the need of some more expensive equipment for example as in the case of ASE or SPE. This method could be a good tool for routine control of food products including confectionery.

### Compliance with Ethical Standards

**Conflict of Interest** Martin Adam declares that he has no conflict of interest. Tomáš Bajer declares that he has no conflict of interest. Petra Bajerová declares that she has no conflict of interest. Karel Ventura declares that he has no conflict of interest.

**Ethical Approval** This article does not contain any studies with human or animal subjects.

**Informed Consent** Informed consent was not applicable

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