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5-HYDROXYMETHYLFURFURAL CONTENT IN FOODSTUFFS DETERMINED BY MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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Abstract

Micellar electrokinetic chromatography (MEKC) has been applied for the determination of 5-hydroxymethylfurfural in several foodstuffs. A 75 mM phosphate buffer solution at pH 8.0 containing 100 mM sodium dodecylsulfate was used as background electrolyte (BGE), and the separation was performed by applying +25 kV in a 50 µm I.D. uncoated fused-silica capillary. Good linearity over the range 2.5-250 mg kg^{-1} ($r^2 \geq 0.999$) and run-to-run and day-to-day precisions at low and medium concentration levels were obtained. Sample limit of detection (0.7 mg kg^{-1}) and limit of quantification (2.5 mg kg^{-1}) were established by preparing the standards in blank matrix. The procedure was validated by comparing the results with those obtained with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Levels of HMF in 45 different foodstuffs such as breakfast cereals, toasts, honey, orange juice, apple juice, jam, coffee, chocolate and biscuits were determined.

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1. Introduction

5-Hydroxymethylfurfural (5-hydroxymethyl-2-furaldehyde, HMF) is one of the most common intermediate products of the well-known Maillard Reaction (Berg & Boekel, 1994; Friedman, 1996; Rufian-Henares, Delgado-Andrade & Morales, 2009; Tomlinson, Landers, Lewis & Naylor, 1993). This complex set of non-enzymatic browning reactions takes place when processing and cooking foods that contain reducing sugars and amino acids. HMF is an early marker of this reaction since it is formed during its initial steps, and occurs in many carbohydrate-rich foods. In addition, this cyclic aldehyde is also produced during hexoses dehydration under acidic conditions via enolisation (Gottschalk, 1952; Kroh, 1994), followed by glucosamine hydrolysis (Jun, Shao, Ho, Koetter & Lech, 2003; Leloir & Cardini, 1953) and it appears naturally in products in which water coexists with monosaccharides in acidic medium. Moreover, HMF is one of the decomposition products of ascorbic acid and it is used to evaluate the severity of heating during fruit-juice processing (Burdurlu, Koca & Karadeniz, 2006). Therefore, HMF is formed not only from the Maillard Reaction, but also from hexoses degradation and caramelisation, for which the presence of amino groups is not needed (Berg et al. 1994).

Although HMF is nearly absent in fresh and untreated foods, its concentration tends to rise during heating, so it is a useful tool to evaluate heat damage in foodstuffs (Fallico, Arena & Zappala, 2003). Moreover, it is also a recognised parameter of food freshness and quality. Hence, the analytical control of HMF has been used in food surveillance to evaluate both the quality of the processing method and the organoleptic characteristics of the final product. In fact, the Codex Alimentarius of the World Health Organisation (Alinorm 01/25, 2001) and the European Union (Directive 110/2001/EC, 2001) have established a maximum HMF quality level in honey (40 mg kg^{-1}) and in apple juice (50 mg L^{-1}) as a deterioration and heat-treatment indicator.

For years, the presence of HMF in foodstuffs has attracted interest because it exhibits mutagenic and DNA strand-breaking activity (Omura, Jahan, Shinohara & Murakami, 1983). HMF in food has also raised toxicological concern since this compound and its derivatives,

5-sulfooxymethylfurfural (SMF) and 5-chloromethylfurfural (CMF), are cytotoxic (Naessberger, 1990), genotoxic (Bruce et al. 1993; Severin, Dumont, Jondeau-Cabaton, Graillot & Chagnon, 2010), nephrotoxic (Bakhiya, Monien, Frank, Seidel & Glatt, 2009), mutagenic and carcinogenic (Lee, Shlyankevich, Jeong, Douglas & Surh, 1995; Monien, Frank, Seidel & Glatt, 2009; Surh, Liem, Miller & Tannenbaum, 1994; Surh & Tannenbaum, 1994; Zhang, Chan, Stamp, Minkin, Archer & Bruce, 1993), inducing colon-rectum, hepatic and skin cancers. In addition, recent publications described a possible carcinogenic potential of HMF due to a metabolic activation by rats and human sulfotransferases (SULT), which transform HMF into its mutagenic derivative SMF (Sommer, Hollnagel, Schneider & Glatt, 2003). Furthermore, some reports have shown HMF to be an in vitro mutagen promoter and initiator of colonic aberrant crypt foci (ACF), which is a biomarker of genotoxicity and carcinogenicity in rat cell lines (Glatt, Schneider & Liu, 2005; Skog & Alexander, 2006; Svendsen, Husoy, Glatt, Haugen & Alexander, 2007). The extrapolation to humans could be more dramatic since humans express SULT in extrahepatic tissues more extensively than rats and may therefore be more sensitive to HMF (Teubner, Meinl, Florian, Kretzschmar & Glatt, 2007). However, in vitro studies on genotoxicity and mutagenicity have given controversial results and the toxicological relevance of its exposure has not been clarified yet since the mechanisms by which HMF exerts its genotoxicity remain unclear (Husoy et al. 2008; Janzowski, Glaab, Samimi, Schlatter & Eisenbrand, 2000).

Several methods have been developed in the past decades to determine HMF in foodstuffs, most of them based on classical spectrophotometric techniques (White, 1979). Liquid chromatography (LC) with UV detection is commonly used for HMF determination and it is regarded as the reference method by the Association of Official Analytical Chemists (AOAC method 980.23, 1996). Recently more selective analytical methods based on mass spectrometry (MS) have been published. LC-MS and LC-MS/MS have been used to analyse HMF in several foodstuffs (Gokmen & Senyuva, 2006; Teixido, Moyano, Santos & Galceran, 2008), and gas

chromatography coupled to mass spectrometry (GC-MS) (Teixido, Santos, Puignou & Galceran, 2006) has also been proposed for HMF determination.

Capillary electrophoresis (CE) has been demonstrated to be a powerful technique for food analysis (Cifuentes, 2006; Garcia-Canas & Cifuentes, 2008) and therefore it can be a promising alternative for the analysis of HMF. Due to the chemical characteristics of this compound (high polarity and difficult ionisation), conventional capillary zone electrophoresis (CZE) cannot be applied. In contrast, micellar electrokinetic chromatography (MEKC), as first introduced by Terabe, Otsuka, Ichikawa, Tsuchiya and Ando (1984), can be a good alternative to LC and GC techniques. The literature is scarce on the determination of heat-induced markers in foodstuffs by electrophorethic techniques. Up to now, only two papers have been published dealing with the determination of HMF by MEKC (Corradini & Corradini, 1992; Morales & Jimenez-Perez, 2001) but few information is provided about its applicability when analysing food samples of different nature. Since those methods are focused only in grape juice and milk-formulae, it is interesting setting up procedures for the analysis of HMF in a wider range of matrices such as breakfast cereals, chocolate and biscuits.

The aim of this work is the application of a MEKC procedure with direct UV detection suitable for the determination of HMF in a wide range of different foodstuffs typically consumed in the Spanish diet. The MEKC method was validated by determining HMF content of representative samples and comparing the results with those obtained using a previously established LC-MS/MS method (Teixido et al. 2008). A total of 45 food commodities obtained from commercial markets, including honeys, fruit juices, biscuits, breakfast cereals, jams, breads, coffees and chocolates were then analysed by MEKC, and the HMF concentration results were discussed and compared to those found by other researchers.

2. Materials and methods

2.1. Chemicals

High purity (>99%) HMF (5-hydroxymethylfurfural) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (HPLC grade), hydrochloric acid (25%) and sodium hydroxide were purchased from Merck (Darmstad, Germany). SDS, sodium dihydrogen phosphate monohydrate and sodium monohydrogen phosphate anhydrous were obtained from Fluka (Buchs, SG, Switzerland). Syringe filters (0.45 μm) were purchased from Teknokroma (Barcelona, Spain) and water was purified by means of an Elix-Milli-Q system (Millipore, Bedford, MA, USA).

A stock standard solution of HMF (1500 mg L^{-1}) was prepared in water. Intermediate working solutions were prepared weekly from the primary standard solution by appropriate dilution in water. Standard solutions ranging from 0.75 mg kg^{-1} to 250 mg kg^{-1} were prepared in a blank matrix (fresh orange juice free of HMF). All stock solutions were stored at 4 ºC for no more than two weeks. Buffers were prepared daily by dilution of stock solutions of 0.5 M sodium hydrogenphosphate and 0.5 M sodium dihydrogenphosphate. Stock solution of 0.5 M SDS was prepared every week in purified water. BGE was prepared every day by dilution of the SDS stock solution in appropriate phosphate buffer. All buffers and working standard solutions were sonicated and filtered through 0.45 μm nylon filters before use.

For solid-phase extraction (SPE), ENV+ cartridges (200 mg, 3 mL) were purchased from IST (Hengoed, Mid-Glamorgan, UK); coupling pieces and stopcocks were obtained from Varian (Harbor City, USA). Nitrogen (99.8% purity) was supplied by a Claind nitrogen generator N2 FLO (Lenno, Italy).

For sample clean-up, clarification and protein precipitation solutions of Carrez I (Potassium hexacyanoferrat(II)-3-hydrat, 150 g/L) and Carrez II (Zincsulphate-7-hydrat, 300 g/L) were prepared in distilled water.

For LC-MS/MS analysis, [2,3,3-$^{2}H_3$]Acrylamide (acrylamide-d_3) (98%) provided by Cambridge Isotope Labs. (Andover, MA, USA) was used as internal standard. Methanol (HPLC grade) was purchased from Merck (Darmstadt, Germany). A stock standard solution of HMF (500 mg kg^{-1}) was prepared by weight in water. Intermediate solutions were prepared weekly from

156 the primary standard solution by appropriate dilution in water. Calibration standard solutions
157 ranging from 0.24 mg kg^{-1} to 65 mg kg^{-1} were prepared in a blank matrix (fresh orange juice free of
158 HMF). An acrylamide-d3 standard solution (1000 mg L^{-1}) was prepared in water and used as the
159 internal standard for LC–MS/MS quantification. All stock solutions were stored at 4 ºC for no more
160 than 2 weeks.
161 All food samples (orange juices, honeys, breakfast cereals, jams, biscuits, toasts, apple juices,
162 coffees, and chocolates as well as oranges for preparing the fresh squeezed orange juice) were
163 purchased at a local market in Barcelona (Spain).
164
165 **2.2. Instrumentation and working conditions**
166 **2.2.1. MEKC**
167 Experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis
168 instrument (Fullerton, CA, USA) equipped with a diode array detection system. Electrophoretic
169 separation was carried out using uncoated fused-silica capillaries (Beckman) with a total length of
170 60 cm (50 cm effective length) and 50 μm I.D. A 75 mM phosphate buffer (pH 8) solution
171 containing 100 mM SDS was used as BGE, and it was filtered through a 0.45μm membrane filter
172 and degassed by sonication before use. A capillary voltage of +25 kV (~110 μA) was applied for
173 the separation, and the temperature was held at 25 ºC. Samples were loaded by hydrodynamic
174 injection pressure assisted (3.5 kPa) during 15 s and direct detection was carried out at 284 nm. The
175 CE instrument was controlled using a Beckman P/ACE station software version 1.2.
176
177 **2.2.2. Capillary conditioning**
178 New capillaries were pre-treated by using 0.1 M hydrochloric acid for 30 min, Milli-Q water
179 for 30 min, 0.1 M sodium hydroxide for 30 min, and finally rinsed with Milli-Q water for 30 min.
180 The capillary was conditioned daily by rinsing with 0.1 M sodium hydroxide for 30 min, Milli-Q

water for 30 min and finally with the BGE for 30 min before the first run. Finally, the capillary was rinsed with BGE for 5 min between runs and stored after rinsing with water.

2.2.3. LC–MS/MS

A previously developed LC-MS/MS method (Teixido et al. 2008) was used in order to validate the MEKC method proposed in the present work. Briefly, a fluorinated narrow-bore LC column Discovery HS F5 (Supelco, Bellefonte, PA, USA) at 40ºC under isocratic elution using H_2O:MeOH 9:1 (v,v) as mobile phase was used. Flow rate was set at 0.2 mL min^{-1} and the injection volume was 10 μL.

A quaternary pump Alliance 2690 system from Waters was coupled to a LCQ mass spectrometer (Thermo Electron, San Jose, CA, USA) equipped with atmospheric pressure chemical ionisation (APCI) source as well as an ion trap as mass analyser. Data acquisition and general operation was carried out via Xcalibur software (version 1.4). APCI working conditions in positive mode were as follows: spray current and discharge voltage were 5 mA and 5 kV, respectively; heated capillary temperature was held at 175 ºC and the vaporiser temperature at 350 ºC; nitrogen was used as sheath gas at a flow rate of 23 arbitrary units (a.u.), and as auxiliary gas at a flow rate of 13 a.u.

Data acquisition was performed in product ion scan of the protonated molecule $[M+H]^+$ (*m*/*z* 127) scanning product ions from *m*/*z* 50 to *m*/*z* 200. The fragment ion at *m*/*z* 109 was used for quantitation purposes. For the internal standard (acrylamide-d3), the precursor ion was *m*/*z* 75 and data acquisition was performed in product ion scan mode (*m*/*z* 50–150) using the most intense product ion (*m*/*z* 58) for quantitative analysis.

2.3. Sample preparation and clean-up procedure

Sample preparation was performed following a previously proposed procedure (Teixido et al. 2008) with some modifications. Briefly, food samples were ground and homogenised using a

supermixer blender system (Moulinex, Lyon, France) and an Ultraturrax T25 basic (Ika-Werke, Staufen, Germany). Sample amounts of 1 g were weighed into 15 mL centrifuge tubes and 9 mL of water were added. Each tube was stirred for 1 min in a Stuart vortex mixer (Barloworld Scientific, Stafford, UK). Then, 0.5 mL of each Carrez Solution (I and II) were added and subsequent centrifugation was performed at 4000 rpm for 5 min with a Selecta Centronic centrifuge (Selecta, Barcelona, Spain). The supernatant aqueous sample solution was filtered through a nylon 0.45 µm syringe filter (Tracer).

Supelco Visiprep and Visidry SPE vacuum manifolds (Supelco, Gland, Switzerland) were used for SPE and solvent evaporation, respectively. Initially, ENV+ cartridges (200 mg, 3 mL) from IST (Hengoed, UK) were conditioned using 5 mL of methanol and 5 mL of water. Five millilitres of the sample aqueous solution was loaded to the cartridge, followed by 1 mL of water in order to wash the cartridge and eliminate any interference. Then cartridges were dried during 1 minute and HMF was eluted with 1 mL of methanol. The resulting extract was evaporated to dryness under a stream of nitrogen, reconstituted in 0.5 mL of water, filtered through a 0.45 µm nylon syringe filter, injected into the CE system and analysed using the proposed MEKC procedure.

3. Results and discussion

3.1. Method performance

Electrophoretic conditions such as buffer concentration, SDS concentration, pH and capillary voltage were slightly modified form those previously published (Corradini et al. 1992) in order to adjust them to our requirements. It was observed that higher pH values provided better HMF signal, without a significant variation on analysis time and pH 8 was selected as the optimal value instead of the value of pH 7.5 proposed by Corradini and co-workers. Improvements on HMF signal and peak shape were observed when increasing the ionic strength of the BGE but a considerably increase on capillary current was obtained which resulted in a loss on reproducibility, so 75 mM was chosen as optimal value for buffer concentration.

In order to improve the signal of HMF different SDS concentrations on the BGE were also evaluated and 100 mM was selected, as suggested in the literature (Corradini et al. 1992). High concentrations of SDS improved the separation between the electroosmotic flow (EOF) and HMF but also produced an enhancement of the ionic strength and, consequently, the capillary current also raised, giving as a result worse signal to noise ratio and lower reproducibility because of the Joule effect. Then, a 75 mM phosphate buffer solution at pH 8.0 containing 100 mM SDS was proposed as optimal BGE.

The effect of the capillary voltage (from 5 to 30 kV) on the HMF determination was also studied. Although the analysis time decreased when increasing the capillary voltage as expected, values higher than 25 kV produced a loss in the resolution between the EOF and HMF and an increase in capillary current. For these reasons +25 kV was chosen as the optimal capillary voltage value. Both hydrodynamic and electrokinetic injection modes were tested for the introduction of the sample, and the best results were obtained with the hydrodynamic one. For hydrodynamic injection, both BGE and water were used as solvents for standards. Since no significant improvement in the signal was obtained when using BGE and some samples were not completely dissolved, standard solutions were prepared in water.

3.2. Quality parameters

In order to check the performance of the proposed method, quality parameters such as limit of detection (LOD), limit of quantification (LOQ), linearity, and run-to-run and day-to-day precisions were established and the results are given in Table 1. Instrumental quality parameters were determined using aqueous standard solutions of HMF while method quality parameters were calculated using a fresh squeezed orange juice (free of HMF) as blank sample (from now on pseudo-matrix matched standards). These samples were spiked at low concentration levels and submitted to the sample treatment detailed in the experimental section. Instrumental LOD and LOQ, based on a signal-to-noise ratio of 3:1 and 10:1 respectively, were found to be 0.3 mg mL^{-1} and 1

mg L^{-1} for standard solutions. Using pseudo-matrix matched standard solutions the LOD and LOQ of the method slightly increased to 0.7 mg kg^{-1} and 2.5 mg kg^{-1} respectively, due to the sample matrix.

Calibration curves based on HMF peak area at the working ranges indicated in Table 1 were obtained and good linearity, with correlation coefficients (r^2) higher than 0.999, was observed in both cases. Run-to-run and day-to-day precisions for HMF quantification were calculated at two concentration levels, a low level (LOQ) and a medium level (40 mg kg^{-1}) which corresponds to the maximum quality level proposed in honey (Directive 110/2001/EC, 2001). In order to obtain the run-to-run precision, six replicate determinations were carried out under optimal conditions, while the day-to-day precision was calculated by performing 18 replicate determinations of the same solution in three non-consecutive days (six replicates each day). The relative standard deviation (RSD) values obtained for run-to-run and day-to-day precisions were 1.1% and 5.8% respectively, at the medium concentration level. These values increased, as expected, to 7.5% and 10.2% when the low concentration levels were evaluated. Although better precisions were obtained at the high concentration level evaluated, the loss in reproducibility when working at low levels was not significant, showing a good MEKC instrumental performance. The RSD values achieved using the developed MEKC-UV method with the standards prepared on squeezed orange juice were obviously slightly higher than those obtained on the instrumental evaluation using aqueous standards, but with values always lower than 12%. Therefore, the results obtained showed that the developed MEKC-UV method can be proposed for the analysis of HMF at the levels recommended by the Codex (Alinorm 01/25, 2001) and the European Union (Directive 110/2001/EC, 2001). Finally, in terms of migration time, good run-to-run and day-to-day precisions were obtained in all cases (Table 1) with RSD values from 1.3% to 2.1% for matrix matched standard solutions.

For quantiation pseudo-matrix matched using a fresh squeezed orange juice free of HMF was used. In order to validate this quantitation procedure several representative food samples were analysed by LC-MS/MS using a method previously developed in our research group (Teixido et al.

2008). One sample of each type of foodstuff was analysed by both methods (MEKC and LC-MS/MS). The results obtained are shown in Figure 1 where it can be seen that they were not significantly different. Furthermore, a statistical paired-sample comparison analysis was performed with the MEKC-UV results and those obtained by LC–MS/MS and the significance value (*P*) (for a confidence level of 95%) obtained was 0.21. As this value is higher than the significant level selected (*P* value of 0.05), no significant differences were statistically observed between the MEKC and the LC-MS/MS method. Consequently, the pseudo-matrix matched standard calibration method proposed can be used to quantify HMF in food samples by MEKC. This procedure allowed reducing the analysis time and the effort that involves the use of the standard addition method for a high number of samples in routine analysis.

Two additional parameters, the decision limit (CCα) and the detection limit (CCβ), included in the Commission Decision 2002/657/EC (2002) were also evaluated in apple juice and honey, where maximum HMF quality levels have been established by the Codex Alimentarius (Alinorm 01/25, 2001) and the European Union (Directive 110/2001/EC, 2001), respectively. For apple juice there is a lack of EU legislation regarding established maximum HMF levels. So, 20 blank samples were fortified at the maximum HMF quality level recommended (50 mg kg^{-1}) and were analysed following the methodology proposed in this work. CCα was then calculated as the level determined plus 1.64-times the standard deviation of the within-laboratory reproducibility (obtained after the quantitation of the 20 fortified blank samples). Following the same procedure, CCβ was calculated as the measured content at CCα plus 1.64-times the standard deviation of the within-laboratory reproducibility). For the case of HMF in apple juice, CCα and CCβ were calculated to be 57.4 and 64.7 mg kg^{-1}, respectively.

For honey, since the EU states a maximum level of HMF, CCα and CCβ were calculated using the calibration curve approach. For this purpose, a honey sample was fortified around the maximum HMF quality level established (40 mg kg^{-1}). Samples were then analysed following the methodology proposed in this work and the standard addition calibration curve was plotted. CCα

was then calculated as the corresponding concentration at the maximum HMF quality level established plus 1.64-times the standard deviation of the within-laboratory reproducibility (obtained from the standard deviation S_{xo} of the standard addition calibration curve). CCβ was then calculated as the measured content at CCα plus 1.64-times the standard deviation of the within-laboratory reproducibility. For HMF in honey, CCα and CCβ resulted to be 43.9 and 47.9 mg kg^{-1}, respectively.

3.3. Analysis of food commodities

To demonstrate the applicability of the proposed MEKC method for the routine determination of HMF in different types of food products, 45 foodstuff samples of different nature (honey, breakfast cereals, orange and apple juice, biscuits, toasts, coffee, chocolate and jam) were analysed. All samples were prepared as described in Section 2 and analysed by triplicate to determine HMF using fresh orange juice to prepare the standards for pseudo-matrix-matched standard calibration. It must be noted that some coffee and toast samples deserved an additional dilution of the extract before MEKC analysis in order to obtain a response able to be quantified within the linear calibration range since they presented a very high HMF content. As an example, Figure 2 shows the electropherograms obtained for some representative samples (one of each type of foodstuff) and the corresponding spiked ones. Peak purity was confirmed by comparing UV spectra through the entire peak signal.

All the results obtained are summarised in Table 2. Moreover, levels of HMF found by other researchers have also been detailed in the table in order to compare our results with those published in the literature. As can be observed, HMF was found at concentration levels between >0.7 mg kg^{-1} and 1093.0 mg kg^{-1} with good precision (RSD lower than 15% in most of the cases). For some samples, such as dark chocolate A, wheat toast A and peach jam, higher RSD values (18-29%) were observed. These RSD values were higher than those obtained during method validation (Table 1), fact that could be explained because only an analysis by triplicate (instead of 6 determinations as in

the case of validation) was performed. Only six samples, corresponding to orange and apple juices, were found to have concentrations lower than LOQ or LOD, remarking the fact that fresh orange juice can be considered as blank sample and therefore it can be used for preparing the matrix matched standards.

While fruit juices presented low levels of HMF, chocolate, toasts and coffee were the matrices that presented the highest HMF content. It should be taken into account that all the samples in which production and manufacture toasting and drying processes are involved, usually present higher HMF amounts since these thermal processes favour both the Maillard Reaction and the caramelisation. Moreover, levels of HMF in instant coffee were significantly higher as compared to natural and blend coffee in agreement with literature data (Arribas-Lorenzo & Morales, 2010).

Regarding biscuits, the sample that presented the highest HMF content was the digestive biscuits, which is due to the presence of glucose syrup that enhances HMF formation when baking. For toasts, slightly higher values were found for whole wheat toasts than for wheat ones. This could be partially explained by the slower rate of water loss of whole-wheat bread as compared with the wheat formulation (Capuano et al. 2009). However, the differences of HMF content observed in the analysed samples can also be attributed to different toasting temperatures both in the case of toasts and biscuits.

Regarding breakfast cereals, rice based cereals have the lowest HMF levels while higher levels correspond to corn and wheat based cereals, in agreement with the literature data (Garcia-Villanova, Guerra-Hernandez, Martinez-Gomez & Montilla, 1993). Obviously, honey biscuits and honey cereals (honey rings) have higher values than the plain ones since honey gives these foodstuffs an extra source of HMF.

4. Conclusions

A MEKC method with pseudo-matrix matched calibration procedure has been proved to be applicable to the analysis of 5-hydroxymethylfurfural in foodstuffs with matrices of different nature and at different concentration levels. Micellar background electrolyte conditions have been adjusted. Sample preparation with an efficient SPE clean-up, followed by a rapid electrophoretic determination is proposed. Good repeatability, linearity and sensitivity were obtained. Moreover, the method was validated by comparing the results with those obtained by LC–MS/MS and no significant differences were observed. The proposed MEKC method was applied for determining HMF in 45 food samples containing a broad range of HMF levels in very different matrices. Therefore, it can be proposed as an alternative to the LC-UV Official Method (AOAC Method 980.23, 1996) for the analysis of HMF in food.

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6. References

Alinorm 01/25 (2001). *Codex Alimentarius*.

Arribas-Lorenzo, G. & Morales, F. J. (2010). Estimation of dietary intake of 5-hydroxymethylfurfural and related substances from coffee to Spanish population. *Food and Chemical Toxicology*, 48(2), 644-649.

Association of Official Analytical Chemists**,** Official Methods of Analysis (1996). *Official Methods of Analysis of AOAC International*, 4426.

Bakhiya, N., Monien, B., Frank, H., Seidel, A., & Glatt, H. (2009). Renal organic anion transporters OAT1 and OAT3 mediate the cellular accumulation of 5-sulfooxymethylfurfural, a reactive,

387 nephrotoxic metabolite of the Maillard product 5-hydroxymethylfurfural. *Biochemical*
388 *Pharmacology*, 78(4), 414-419.

389 Berg, H. E. & Boekel, M. A. J. S. (1994). Degradation of lactose during heating of milk. 1.
390 Reaction pathways. *Netherlands Milk and Dairy Journal*, 48(3), 157-175.

391 Bruce, W. R., Archer, M. C., Corpet, D. E., Medline, A., Minkin, S., Stamp, D., Yin, Y., & Zhang,
392 X. M. (1993). Diet, aberrant crypt foci and colorectal cancer. *Mutation Research*, 290(1), 111-118.

393 Burdurlu, H. S., Koca, N., & Karadeniz, F. (2006). Degradation of vitamin C in citrus juice
394 concentrates during storage. *Journal of Food Engineering*, 74(2), 211-216.

395 Capuano, E., Ferrigno, A., Acampa, I., Serpen, A., Acar, O. C., Goekmen, V., & Fogliano, V.
396 (2009). Effect of flour type on Maillard reaction and acrylamide formation during toasting of bread
397 crisp model systems and mitigation strategies. *Food Research International*, 42(9), 1295-1302.

398 Cifuentes, A. (2006). Recent advances in the application of capillary electromigration methods for
399 food analysis. *Electrophoresis*, 27(1), 283-303.

400 Corradini, D. & Corradini, C. (1992). Separation and determination of 5-hydroxymethyl-2-
401 furaldehyde and 2-furaldehyde in fruit juices by micellar electrokinetic capillary chromatography
402 with direct sample injection. *Journal of Chromatography*, 624(1-2), 503-509.

403 Delgado-Andrade, C., Rufian-Henares, J. A., & Morales, F. J. (2009). Hydroxymethylfurfural in
404 commercial biscuits marketed in Spain. *Journal of Food and Nutrition Research*, 48(1), 14-19.

405 Directive 2001/110/EC of 20 December 2001. *Official Journal of the European Communities*, 47-
406 52.

407 Directive 2002/657/EC of 12 August 2002. *Official Journal of the European Communities*.

Fallico, B., Arena, E., & Zappala, M. (2003). Roasting of hazelnuts. Role of oil in colour development and hydroxymethylfurfural formation. *Food Chemistry*, 81(4), 569-573.

Friedman, M. (1996). Food Browning and Its Prevention: An Overview. *Journal of Agricultural and Food Chemistry*, 44(3), 631-653.

Garcia-Canas, V. & Cifuentes, A. (2008). Recent advances in the application of capillary electromigration methods for food analysis. *Electrophoresis*, 29(1), 294-309.

Garcia-Villanova, B., Guerra-Hernandez, E., Martinez-Gomez, E., & Montilla, J. (1993). Liquid chromatography for the determination of 5-(hydroxymethyl)-2-furaldehyde in breakfast cereals. *Journal of Agricultural and Food Chemistry*, 41(8), 1254-1255.

Gaspar, E. M. S. M. & Lucena, A. F. F. (2009). Improved HPLC methodology for food control - furfurals and patulin as markers of quality. *Food Chemistry*, 114(4), 1576-1582.

Glatt, H., Schneider, H., & Liu, Y. (2005). V79-hCYP2E1-hSULT1A1, a cell line for the sensitive detection of genotoxic effects induced by carbohydrate pyrolysis products and other food-borne chemicals. *Mutation Research*, 580(1-2), 41-52.

Gokmen, V. & Senyuva, H. Z. (2006). Improved Method for the Determination of Hydroxymethylfurfural in Baby Foods Using Liquid Chromatography-Mass Spectrometry. *Journal of Agricultural and Food Chemistry*, 54(8), 2845-2849.

Gottschalk, A. (1952). *Biochemistry Journal*, 52, 455.

Husoy, T., Haugen, M., Murkovic, M., Joebstl, D., Stolen, L. H., Bjellaas, T., Ronningborg, C., Glatt, H., & Alexander, J. (2008). Dietary exposure to 5-hydroxymethylfurfural from Norwegian food and correlations with urine metabolites of short-term exposure. *Food and Chemical Toxicology*, 46(12), 3697-3702.

Janzowski, C., Glaab, V., Samimi, E., Schlatter, J., & Eisenbrand, G. (2000). 5-Hydroxymethylfurfural: assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. *Food and Chemical Toxicology*, 38(9), 801-809.

Jun, M., Shao, Y., Ho, C. T., Koetter, U., & Lech, S. (2003). Structural identification of non volatile dimerization products of glucosamine by Gas Chromatography-Mass Spectrometry, Liquid Chromatography-Mass Spectrometry, and Nuclear Magnetic Resonance Analysis. *Journal of Agricultural and Food Chemistry*, 51(21), 6340-6346.

Kroh, L. W. (1994). Caramelization in food and beverages. *Food Chemistry*, 51(4), 373-379.

Lee, Y. C., Shlyankevich, M., Jeong, H. K., Douglas, J. S., & Surh, Y. J. (1995). Bioactivation of 5-hydroxylmethyl-2-furaldehyde to an electrophilic and mutagenic allylic sulfuric acid ester. *Biochemical and Biophysical Research Communications*, 209(3), 996-1002.

Leloir, L. F. & Cardini, C. E. (1953). The biosynthesis of glucosamine. *Biochimica Biophysica Acta*, 12(1-2), 15-22.

Mochizuki, N., Hoshino, M., Suga, K., & Sugita-Konishi, Y. (2009). Identification of an interfering substrate in apple juice and improvement for determination of patulin with high-performance liquid chromatography analyses. *Journal of Food Protection*, 72(4), 805-809.

Monien, B. H., Frank, H., Seidel, A., & Glatt, H. (2009). Conversion of the common food constituent 5-hydroxymethylfurfural into a mutagenic and carcinogenic sulfuric acid ester in the mouse in vivo. *Chemical research in toxicology*, 22(6), 1123-1128.

Morales, F. J. & Jimenez-Perez, S. (2001). Hydroxymethylfurfural determination in infant milk-based formulas by micellar electrokinetic capillary chromatography. *Food Chemistry*, 72(4), 525-531.

452 Murkovic, M. & Pichler, N. (2006). Analysis of 5-hydroxymethylfurfual in coffee, dried fruits and
453 urine. *Molecular Nutrition & Food Research*, 50(9), 842-846.

454 Naessberger, L. (1990). Influence of 5-hydroxymethylfurfural (5-HMF) on the overall metabolism
455 of human blood cells. *Human & Experimental Toxicology*, 9(4), 211-214.

456 Nozal, M. J., Bernal, J. L., Toribio, L., Jimenez, J. J., & Martin, M. T. (2001). High-performance
457 liquid chromatographic determination of methyl anthranilate, hydroxymethylfurfural and related
458 compounds in honey. *Journal of Chromatography A*, 917(1-2), 95-103.

459 Omura, H., Jahan, N., Shinohara, K., & Murakami, Y. (1983). Formation of mutagens by the
460 Maillard reaction. In Waller, G.R. and Feather, M.S. *The Maillard Reaction in foods and nutrition.*
461 *American Chemical Society*, 537-563.

462 Rada-Mendoza, M., Olano, A., & Villamiel, M. (2002). Determination of hydroxymethylfurfural in
463 commercial jams and in fruit-based infant foods. *Food Chemistry*, 79(4), 513-516.

464 Rada-Mendoza, M., Sanz, M. L., Olano, A., & Villamiel, M. (2004). Formation of
465 hydroxymethylfurfural and furosine during the storage of jams and fruit-based infant foods. *Food*
466 *Chemistry*, 85(4), 605-609.

467 Ramirez-Jimenez, A., Garcia-Villanova, B., & Guerra-Hernandez, E. (2000).
468 Hydroxymethylfurfural and methylfurfural content of selected bakery products. *Food Research*
469 *International*, 33(10), 833-838.

470 Ramirez-Jimenez, A., Garcia-Villanova, B., & Guerra-Hernandez, E. (2001). Effect of toasting time
471 on the browning of sliced bread. *Journal of the Science of Food and Agriculture*, 81(5), 513-518.

472 Rufian-Henares, J. A. & Delgado-Andrade, C. (2009). Effect of digestive process on Maillard
473 reaction indexes and antioxidant properties of breakfast cereals. *Food Research International*,
474 42(3), 394-400.

Rufian-Henares, J. A., Delgado-Andrade, C., & Morales, F. J. (2009). "Assessing the Maillard reaction development during the toasting process of common flours employed by the cereal products industry". *Food Chemistry*, 114(1), 93-99.

Severin, I., Dumont, C., Jondeau-Cabaton, A., Graillot, V., & Chagnon, M. C. (2010). Genotoxic activities of the food contaminant 5-hydroxymethylfurfural using different in vitro bioassays. *Toxicology Letters*, 192(2), 189-194.

Skog, K., Alexander, J., & Editors. (2006). Acrylamide and other Hazardous Compounds in Heat-Treated Foods. Woodhead Publishing Ltd., Cambridge, UK.

Sommer, Y., Hollnagel, H., Schneider, H., & Glatt, H. R. (2003). Metabolism of 5-hydroxymethyl-2-furfural (HMF) to the mutagen 5-sulfoxymethyl-2-furfural (SMF) by individual sulfotransferases. *Naunyn-Schmiedebergs Archives of Pharmacology*, 367R166-R166.

Spano, N., Casula, L., Panzanelli, A., Pilo, M. I., Piu, P. C., Scanu, R., Tapparo, A., & Sanna, G. (2006). An RP-HPLC determination of 5-hydroxymethylfurfural in honey: The case of strawberry tree honey. *Talanta*, 68(4), 1390-1395.

Surh, Y. J., Liem, A., Miller, J. A., & Tannenbaum, S. R. (1994). 5-Sulfooxymethylfurfural as a possible ultimate mutagenic and carcinogenic metabolite of the Maillard reaction product, 5-hydroxymethylfurfural. *Carcinogenesis*, 15(10), 2375-2377.

Surh, Y. J. & Tannenbaum, S. R. (1994). Activation of the Maillard reaction product 5-(hydroxymethyl)furfural to strong mutagens via allylic sulfonation and chlorination. *Chemical research in toxicology*, 7(3), 313-318.

Svendsen, C., Husoy, T., Glatt, H., Haugen, M., & Alexander, J. (2007). 5-Sulfooxymethylfurfural (SMF), the metabolite of 5-hydroxymethylfurfural (HMF), increases the numbers of adenoma and aberrant crypt foci in the intestine of min-mice. *Toxicology Letters*, 172(Supplement 1), S202.

Teixido, E., Santos, F. J., Puignou, L., & Galceran, M. T. (2006). Analysis of 5-hydroxymethylfurfural in foods by gas chromatography-mass spectrometry. *Journal of Chromatography A*, 1135(1), 85-90.

Teixido, E., Moyano, E., Santos, F. J., & Galceran, M. T. (2008). Liquid chromatography multi-stage mass spectrometry for the analysis of 5-hydroxymethylfurfural in foods. *Journal of Chromatography A*, 1185(1), 102-108.

Terabe, S., Otsuka, K., Ichikawa, K., Tsuchiya, A., & Ando, T. (1984). Electrokinetic separations with micellar solutions and open-tubular capillaries. *Analytical Chemistry*, 56(1), 111-113.

Teubner, W., Meinl, W., Florian, S., Kretzschmar, M., & Glatt, H. (2007). Identification and localization of soluble sulfotransferases in the human gastrointestinal tract . *Biochemical Journal*, 404(2), 207-215.

Tomlinson, A. J., Landers, J. P., Lewis, I. A. S., & Naylor, S. (1993). Buffer conditions affecting the separation of Maillard reaction products by capillary electrophoresis. *Journal of Chromatography A*, 652(1), 171-177.

Vorlova, L., Borkovcova, I., Kalabova, K., & Vecerek, V. (2006). Hydroxymethylfurfural contents in foodstuffs determined by HPLC method. *Journal of Food and Nutrition Research*, 45(1), 34-38.

White, J. W. J. (1979). Spectrophotometric method for hydroxymethylfurfural in honey. *Journal of AOAC International*, 62(3), 509-526.

Yuan, J. P. & Chen, F. (1998). Separation and Identification of furanic compounds in fruit juices and drinks by High-Performance Liquid Chromatography Photodiode Array Detection. *Journal of Agricultural and Food Chemistry*, 46(4), 1286-1291.

Zhang, X. M., Chan, C. C., Stamp, D., Minkin, S., Archer, M. C., & Bruce, W. R. (1993). Initiation and promotion of colonic aberrant crypt foci in rats by 5-hydroxymethyl-2-furaldehyde in thermolyzed sucrose. *Carcinogenesis*, 14(4), 773-775.

Figure Captions

Figure 1. Comparison of the MEKC-UV quantitation results with those obtained for the same samples by LC-MS/MS.

Figure 2. MEKC-UV electropherograms of one representative sample of each type. BGE: 75 mM phosphate buffer at pH 8.0 containing 100 mM sodium dodecylsulfate. Capillary voltage, +25 KV. Acquisition wavelength, 284 nm. Hydrodynamic injection 10s (3.5 KPa)
(a) original sample (b) spiked sample (20 mg kg^{-1})

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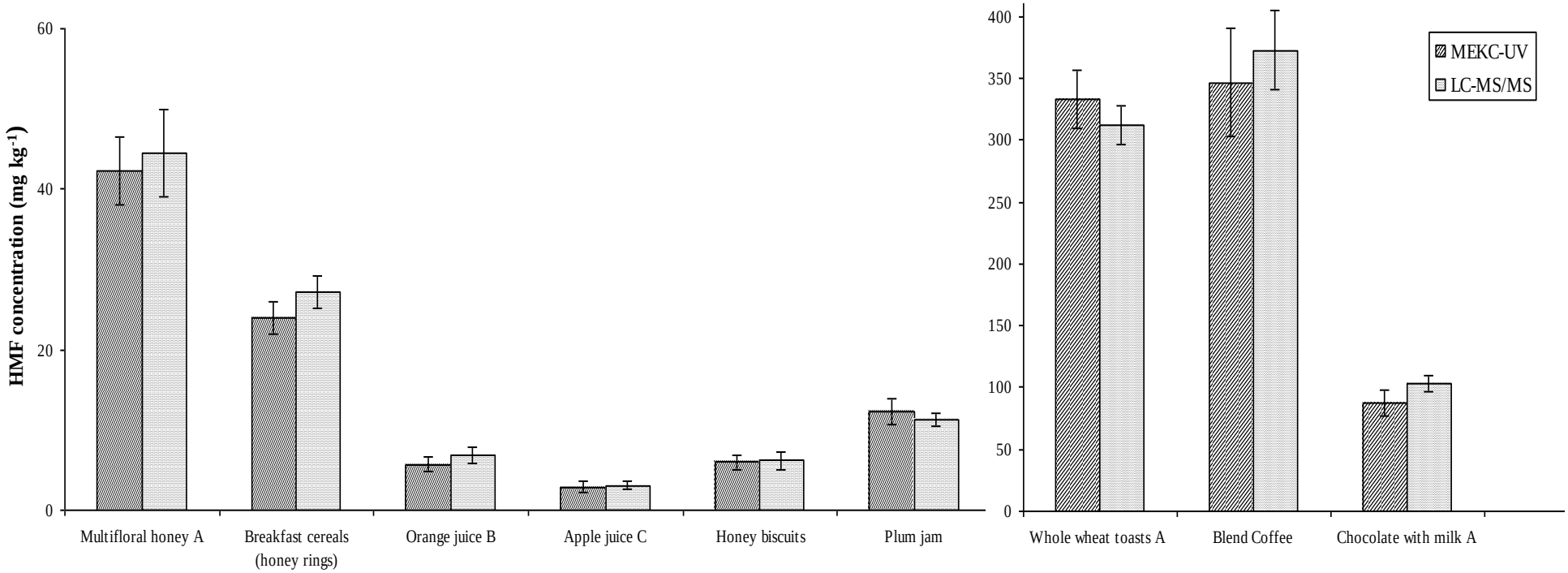
Figure 1



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558 Figure 2

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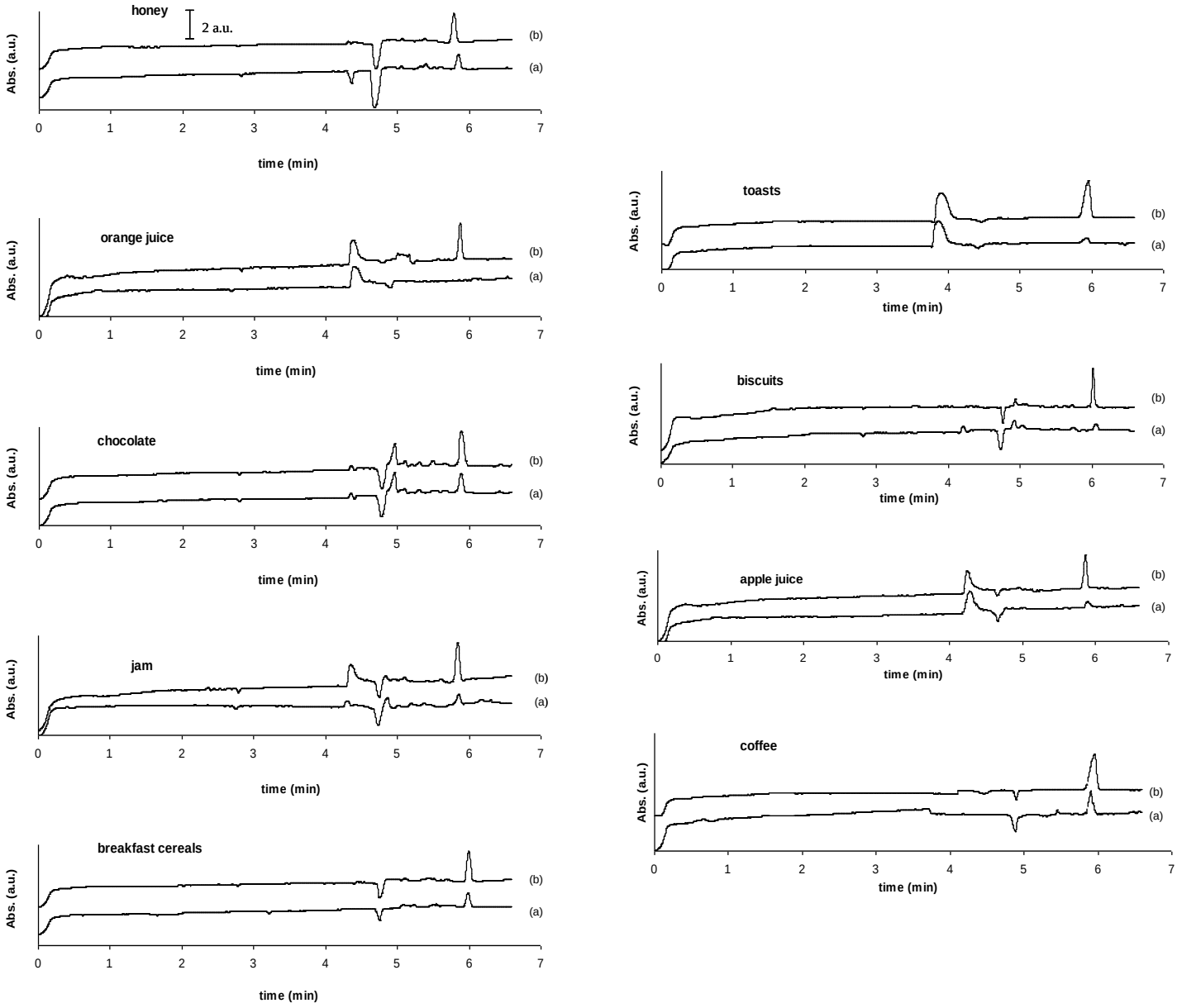

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