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Improved Sample Preparation to Determine Acrylamide in Difficult Matrixes Such as Chocolate Powder, Cocoa, and Coffee by Liquid Chromatography Tandem Mass Spectroscopy

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An improved sample preparation (extraction and cleanup) is presented that enables the quantification of low levels of acrylamide in difficult matrixes, including soluble chocolate powder, cocoa, coffee, and coffee surrogate. Final analysis is done by isotope-dilution liquid chromatography-electrospray ionization tandem mass spectrometry (LC-MS/MS) using d_3-acrylamide as internal standard. Sample pretreatment essentially encompasses (a) protein precipitation with Carrez I and II solutions, (b) extraction of the analyte into ethyl acetate, and (c) solid-phase extraction on a Multimode cartridge. The stability of acrylamide in final extracts and in certain commercial foods and beverages is also reported. This approach provided good performance in terms of linearity, accuracy and precision. Full validation was conducted in soluble chocolate powder, achieving a decision limit (CCα) and detection capability (CCβ) of 9.2 and 12.5 μg/kg, respectively. The method was extended to the analysis of acrylamide in various foodstuffs such as mashed potatoes, crisp bread, and butter biscuit and cookies. Furthermore, the accuracy of the method is demonstrated by the results obtained in three inter-laboratory proficiency tests.

KEYWORDS: Acrylamide; analysis; liquid−liquid extraction; LC-MS/MS; cocoa; chocolate; coffee; chicory; proficiency testing

INTRODUCTION

Since the discovery of acrylamide in foods was made public in April 2002 (*1*), several research groups were involved in rapidly developing methods to reliably quantify acrylamide at relatively low levels in a large variety of different foodstuffs. Most of the methods published so far are based on either GC-MS (*2*−*6*) or LC-MS (*7*−*14*) techniques, with comparable performance of the two approaches. In fact, an excellent review of the different analytical techniques employed to determine acrylamide in foods has recently been published by the European Commission's (EC) Joint Research Centre (JRC) in Geel, Belgium (*15*). Incorporated in the review by Wenzl et al. is detailed information on the sample pretreatment steps used by the laboratories (e.g., extraction conditions, derivatization, cleanup, chromatographic conditions, mass spectrometry parameters). However, based on conclusions of a recent inter-laboratory trial (*16*) and EC/JRC expert workshop held in April 2003 (*17*), many of the methods do not perform well in difficult matrixes such as cocoa and coffee. Consequently, laboratories are continuously adapting their methods to achieve the required precision and sensitivity for those foods in which gaps in the analytical science have been identified.

Only very recently has a single method been reported (*12*) that can achieve good sensitivity and selectivity of acrylamide for practically all of the relevant food matrixes. However, the authors also highlight that coffee is a troublesome matrix, and the selected mass transitions reveal difficulty of obtaining baseline separation in certain profiles (*12*). In addition, multiple responses are observed in each of the mass transitions at different retention times but close to that of acrylamide, which may cause interference.

Our laboratory has recently reported a method that achieves good precision, accuracy, and reliability of indentification for the analyte in a wide range of foodstuffs. However, it was mentioned that acrylamide could not be quantified reliably in difficult matrixes such as cocoa powder and coffee, mainly due to considerable loss of the analyte throughout the sample preparation steps (*14*). We now report on the improvements made to our existing method, which are essentially limited to modifications in the sample extraction and cleanup, providing a common base for the determination of acrylamide in all relevant foods.

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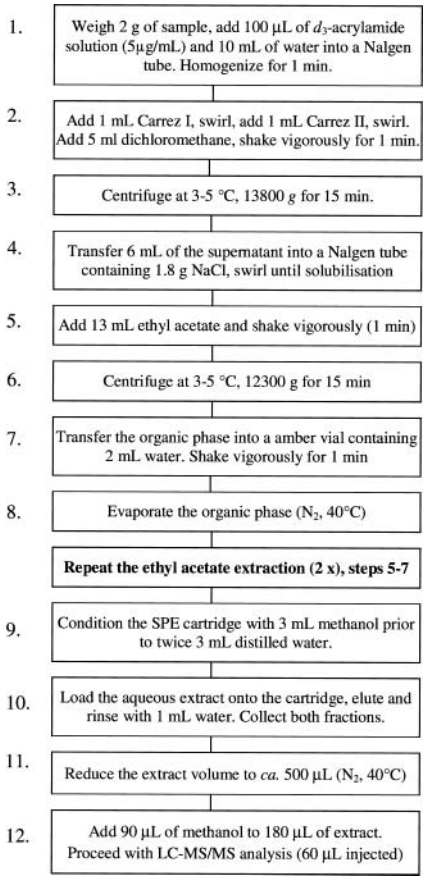

Figure 1. Flowchart of the extraction/cleanup procedure for the analysis of acrylamide in foodstuffs.

MATERIALS AND METHODS

Chemicals. Acrylamide (purity 99%) was supplied by Aldrich (Buchs, Switzerland). Dichloromethane, ethyl acetate, methanol, sodium chloride and formic acid were purchased from Merck (Darmstadt, Germany). Potassium hexacyanoferrate(II) trihydrate and zinc sulfate heptahydrate were obtained from Aldrich (Buchs, Switzerland). d_3−Acrylamide (2,3,3-d_3-2-propenamide) was purchased from Cambridge Isotope Laboratories (Andover, MA).

Food Samples. The food samples were purchased off-the-shelf from retail outlets in Switzerland and stored at room temperature. The full validation was done on a chocolate powder containing 18% cocoa powder.

Preparation of Standards. Aqueous stock solutions of acrylamide and d_3-acrylamide were prepared at concentrations at 50 and 5 μg/mL, respectively. The acrylamide solution was further diluted in water to obtain the matrix-equivalent levels: 0, 10, 50, 200, 500, and 2500 μg/kg. The matrix-equivalent concentration of d_3-acrylamide was 250 μg/kg.

Sample Preparation. A sample portion of 2.0 g was weighed into a Nalgen tube (Verrerie de Carouge, Carouge, Switzerland), a solution containing d_3-acrylamide (100 μL of 5 μg/mL) was added, and the mixture was dispersed in 10 mL of water at 60 °C (**Figure 1**). The mixture was then homogenized with an Ultra-Turrax T25 basic device (Ika-Werke, Staufen, Germany) for 1 min. Visual protein precipitation was achieved within <1 min by addition of 1 mL of a 0.68 M potassium hexacyanoferrate(II) trihydrate solution (Carrez I) and 1 mL of a 2 M zinc sulfate heptahydrate solution (Carrez II) that were added under continuous swirling (1−2 min). Dichloromethane (5 mL) was added, and the resulting solution was stirred for 1 min prior to centrifugation at 10 000 rpm (13800*g*, 15 min, 4 °C). Sodium chloride (1.8 g) was added to a 6-mL fraction of the aqueous phase. Ethyl acetate (13 mL) was added to the aqueous phase, and the resulting suspension was stirred vigorously for 1 min. Phase separation was achieved by a 10 min centrifugation at 3 000 rpm (12300*g*, 4 °C). The organic phase was transferred into a vial containing 2 mL of water, and the mixture was stirred vigorously for 1 min. The temperature was raised to 40 °C, and the ethyl acetate layer evaporated under a gentle stream of nitrogen. Two further extractions of the 6 mL aqueous solution (2 × 13 mL) with ethyl acetate were conducted. The resulting aqueous solution (volume was adjusted with water to ca. 2 mL) was loaded onto a 500 mg-Isolute Multimode cartridge (International Sorbent Technology, Glamorgan, United-Kingdom), preconditioned with first methanol (3 mL) and then water (6 mL). The aqueous solution was eluted and collected. A 1-mL aliquot of water was loaded onto the cartridge, eluted, collected, and mixed with the previous fraction. The extract was concentrated to 500 μL under a gentle stream of nitrogen (40 °C) in a thermal-controlled heater block, which takes approximately 1 h. It was then filtered through a 0.2-μm pore sized Spartan 13/0.2RC cellulose filter (Schleicher & Schnell, Dassel, Germany). An aliquot (180 μL) was mixed with 90 μL of methanol prior to analysis by LC-MS/MS (1.33 g matrix equiv/mL of extract).

LC-MS/MS Conditions. LC-MS/MS analyses were essentially performed as described in Riediker and Stadler (*14*) and will not be elaborated further. The only salient differences to the method described here are that the LC solvent now constituted methanol/water/formic acid 30/70/0.007 (v/v/v) with a flow rate set at 0.6 mL/min, and the polymethacrylate gel column (Shodex RSpak DE−413L) was of smaller diameter and longer, thus achieving better resolution (250 × 4.6 mm i.d., Showa Denko, Tokyo, Japan). The mobile phase was postcolumn diverted to achieve a 0.3 mL/min flow rate in the MS interface. The typical retention time of acrylamide was 6.5 min, and the total run time was 12 min. The injection volume was set at 60 μL.

Method Validation. The method was validated according to EC criteria (*18*), and a full validation was performed with a soluble chocolate powder sample. For roasted and soluble coffee, coffee surrogate, and cocoa, the suitability of the method was determined by supplementing the samples with a known amount of acrylamide. The performance characteristics chosen were linearity, trueness, repeatability (within-day precision), intermediate precision (between-day precision), and loss of the analyte throughout the sample preparation steps (solvent extraction, cleanup). Both the decision limit (CCα) and detection capability (CCβ) were determined in a soluble chocolate powder. CCα refers to the value at which it can be decided that the sample contains acrylamide with an error probability of $\alpha = 1\%$. CCβ is defined as the smallest level of acrylamide that may be identified and quantified in a sample with an error probability of $\beta = 5\%$ (*19*). The determination of CCα and CCβ was adapted from Jülicher et al. (*20*). A soluble chocolate powder sample was fortified at 15, 30, and 45 μg/kg (triplicate for each level), and a linear regression model was applied (response as a function of concentration) to determine the *Y*-intercept. This was repeated 4 days to estimate the standard deviation ($SD_{Y-intercept}$) of the *Y*-intercept. The CCα was defined as the concentration (linear regression with the whole points) corresponding to the *Y*-intercept value + 2.33 × $SD_{Y-intercept}$ and CCβ as the concentration corresponding to the *Y*-intercept value + 3.97 × $SD_{Y-intercept}$ (*21*).

Each day, a calibration line was prepared in water and a set of soluble chocolate powder samples was processed after acrylamide supplementations to achieve three levels, namely 12.7, 304.7, and 2504 μg/kg. At day 1, six replicates were prepared per level, whereas only three were prepared for days 2, 3, and 4. The linearity was controlled by calculating the coefficient of variation (CV = 100 × standard deviation/mean) of the response factor of the calibration line. The trueness was expressed as the deviation = 100 × (measured value − expected value)/expected value. The CV was used to calculate the repeatability with the data of day 1. The intermediate precision was calculated by using the variance of the four mean concentrations obtained at days 1, 2, 3, and 4 minus the variance of the within-day mean (day 1). At day 2, the analyte loss was evaluated at each level (vide supra) by supplementing d_3-acrylamide either in the soluble chocolate powder or the final extract. With *Ratio* = acrylamide response/d_3-acrylamide response, the normalized recovery was calculated as 100 × ($Ratio_{suppl\ extract}/Ratio_{suppl\ powder}$). To ascertain the detection, three transitions were monitored for acrylamide and two for d_3-acrylamide. The relative ion intensities were calculated with the calibration points, and the acceptance criteria for the samples were chosen according to the EC requirements (*18*). To complete the

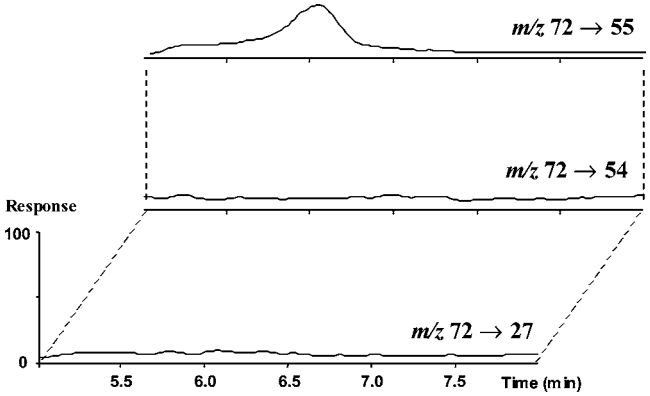

Figure 2. SRM chromatographic profile of the blank chocolate powder used for the validation.

validation, the stability of acrylamide was investigated in soluble chocolate powder extracts and several foodstuffs (breakfast cereal, roasted barley, dried and roasted chicory, roasted and soluble coffee, soluble chocolate powder, chocolate with almonds, and cocoa).

Quantification and Reliability of Identification. In the collision-induced dissociation spectrum of acrylamide, the most intense fragment ion was observed at *m*/*z* 55 (*14*), leading to the highest response for transition *m*/*z* 72 → 55 in the chromatographic profile of a standard when compared to transitions *m*/*z* 72 → 54 and *m*/*z* 72 → 27. Therefore, the transition *m*/*z* 72 → 55 was used for quantification (quantifier) in most of the foodstuffs studied, and the transitions *m*/*z* 72 → 54 and *m*/*z* 72 → 27 served as qualifier ions. However, in some coffee, cocoa, and soluble chocolate powder samples, the occasional interference of a compound eluting approximately 30 s before acrylamide in the trace *m*/*z* 72 → 55 was observed (**Figure 2**). In this case, the transition *m*/*z* 72 → 27 was selected as the quantifier trace and transitions *m*/*z* 72 → 55 and *m*/*z* 72 → 54 as qualifiers.

RESULTS AND DISCUSSION

Sample Preparation. Our laboratory previously reported on a LC-MS/MS-based method to quantify acrylamide in various foodstuffs such as breakfast cereals, crackers, biscuits, crisp bread, wafers, cocoa liquor and nuts (*14*). The sample preparation included the extraction of the sample portion with water followed by precipitation of matrix constituents with acetonitrile. Then, a two-step cleanup approach with Isolute Multimode and cation-exchange columns was employed. However, satisfactory chromatograms could not be obtained when the procedure was applied to either coffee or cocoa powder. In these matrixes, the loss of the analyte was >95%, and a significant ion suppression effect led to a low response of acrylamide under positive electrospray ionization conditons.

The challenge in this study was therefore to ameliorate the recovery of acrylamide throughout the sample pretreatment and concomitantly reduce the amount of co-extractives in difficult matrixes. Some different commercially available solid-phase extraction (SPE) cartridges such as reversed phase (Chromabond C18), silica gel (Bakerbond SiOH) or hydrophilic−lipophilic balanced copolymer (Oasis HLB) were tested, but none led to a significant improvement of the chromatographic profile. Therefore, efforts were focused on a combination of liquid−liquid plus SPE. Ethyl acetate appeared to be a suitable solvent to "salt-out" acrylamide from the aqueous mixture. Due to the hydrophilic nature of acrylamide, the analyte was extracted three times with ethyl acetate (volumetric ratio ethyl acetate/water = 3 × 2.2), which achieved a satisfactory extraction yield. The organic solvent was removed under a flow of nitrogen while maintaining a biphasic system, thereby avoiding an intermediary drying step and quantitatively transferring acrylamide back into the aqueous phase ready for the final SPE step.

However, ethyl acetate extraction alone was not adequate in terms of eliminating undesirable co-extractives and was therefore preceded by a precipitation of matrix constituents with Carrez I and II solutions plus a dichloromethane cleanup step (no acrylamide was detected in the organic phase). The selected reaction monitoring (SRM) traces depicted in **Figure 3** corroborate the significant chromatographic improvement that was achieved for cocoa powder.

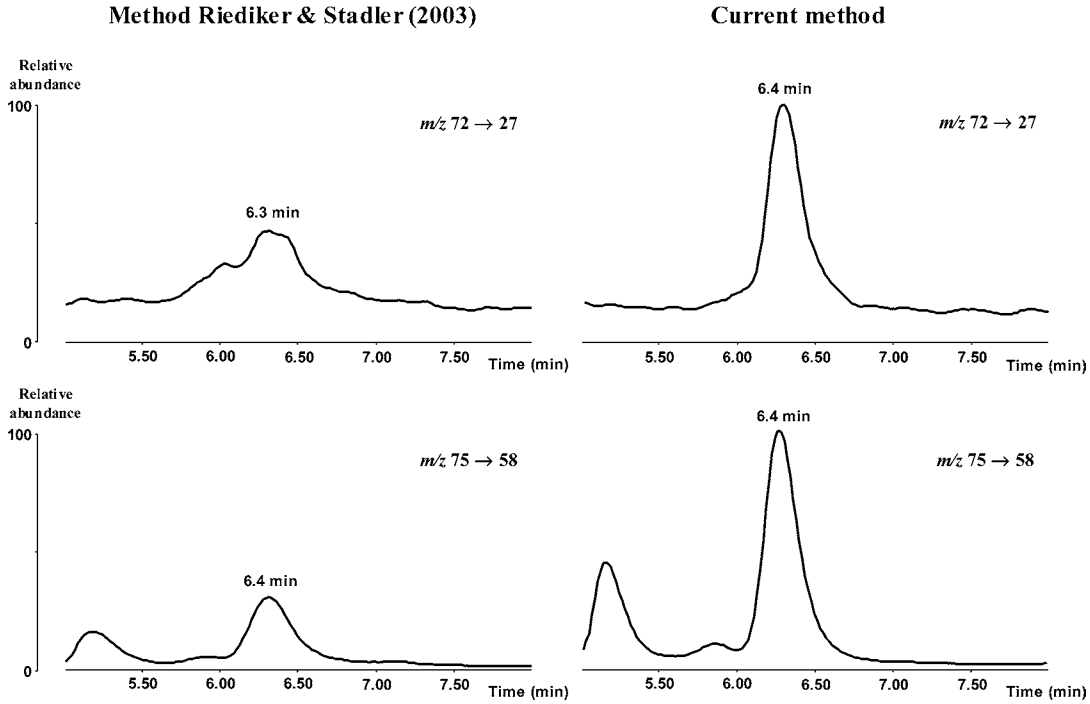

Figure 3. SRM chromatographic profiles of a 168 μg/kg-incurred cocoa prepared and analyzed with either the original method (Riediker and Stadler, 2003) or the modified method presented in this report (only the transitions used for quantification are shown).

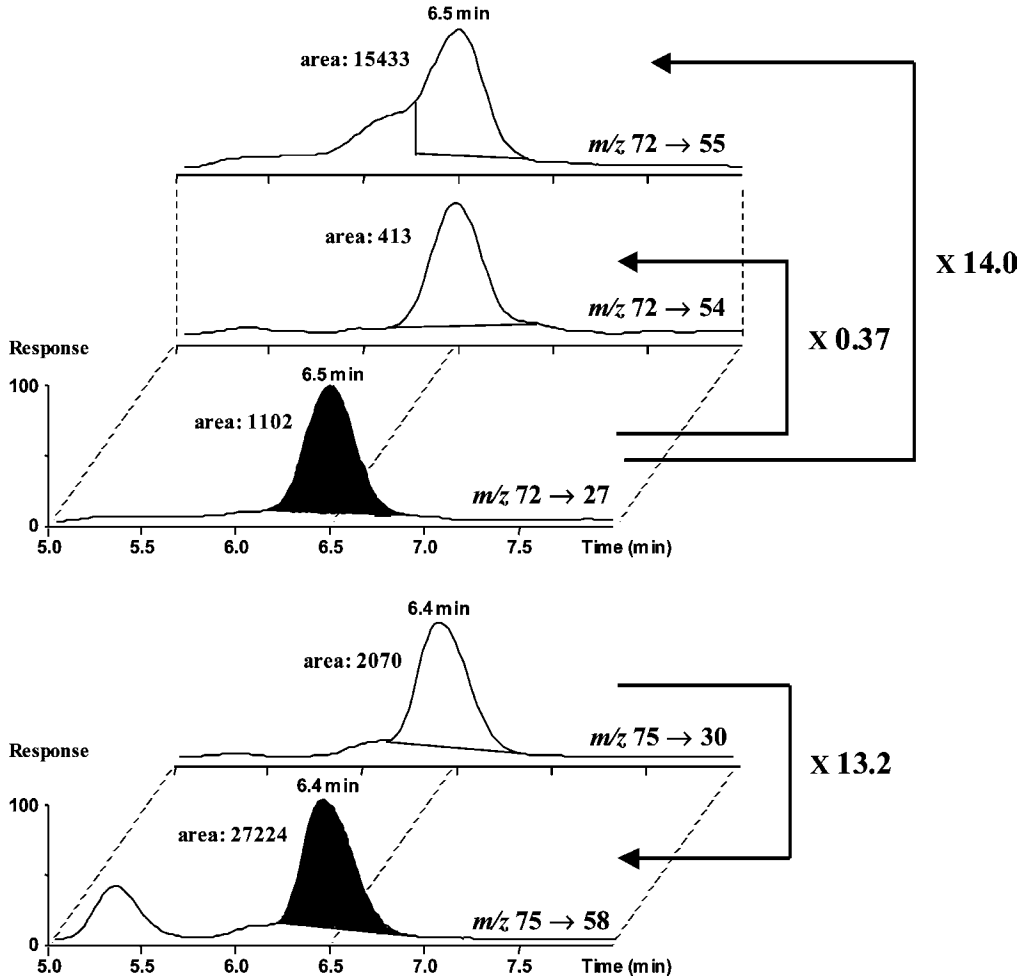

Figure 4. SRM chromatographic profile of an incurred soluble chocolate powder with an acrylamide level measured at 130.0 μg/kg. Ratios between quantifiers (shaded peaks) and qualifiers are indicated. In the calibration points, the mean ratios were: *m/z* 72 → 27*Im/z* 72 → 54 = 0.35 and *m/z* 72 → 55*Im/z* 72 → 27 = 18.7.

Table 1. Performance of the LC-MS/MS Method for the Quantification of Acrylamide in Soluble Chocolate Powder

trueness (deviation in %)	day 1	day 2	day 3	day 4
12.7 μg/kg	7.2	9.0	–24.7	–5.8
304.7 μg/kg	10.7	–5.4	–0.2	–5.7
2504 μg/kg	3.9	–8.3	–0.9	–6.1

precision (CV in%)	repeatability	intermediate precision
12.7 μg/kg	15.8	22.8
304.7 μg/kg	6.1	9.8
2504 μg/kg	5.4	7.7

extractability (%)	12.7 μg/kg	304.7 μg/kg	2504 μg/kg
mean ± SD	42.9 ± 5.9	54.4 ± 2.5	51.1 ± 3.3

CCα	CCβ
9.2μg/kg	12.5μg/kg

Performance of the Method. Acrylamide exhibited good linearity in the calibration points (10.0−2500 μg/kg), and the CV of the response factor was calculated as 3.4, 14.7, 4.9, and 3.2% at days 1, 2, 3, and 4, respectively. The trueness values confirmed the linear response of acrylamide in soluble chocolate powder extracts (**Table 1**). For days 1, 2, and 4, the trueness never exceeded ± 15%. Only at day 3, it was measured to be −24.7% at a concentration of 12.7 μg/kg. Both repeatability and intermediate precision were found below 10% (304.7 and 2504 μg/kg). At a fortification level of 12.7 μg/kg, the repeatability was 15.8% and the intermediate precision 22.8%. The results showed that the extractability of acrylamide from soluble chocolate powder was rather constant along the studied concentration range. However, at the lowest amount (12.7 μg/kg) it seemed to slightly decrease, which may explain, at least in part, the slight loss of precision observed at this low level. The CCα and CCβ for acrylamide in soluble chocolate powder were determined at 9.2 and 12.5 μg/kg, respectively. In our initial work (*14*), we used the terms LOD and LOQ that are defined as S/N = 3 and 10, respectively. Indeed, these definitions are reliable for methods without a time-dependent variation of the response factor. In mass spectrometry, and particularly electrospray ionization mass spectrometry, the ionization yield is not constant and a certain degree of between-sample variation (even with standards) may alter the S/N. In this case, a statistical evaluation appears more suitable, and the definitions of CCα and CCβ, initially established by the regulatory community, are powerful tools to define the performance of a mass spectrometry-based method near the LOD and LOQ. We therefore decided to estimate CCα and CCβ for solely statistical reasons, without any referral to or suggestion of possible future regulatory decisions for this compound.

The multiple-transition recording of both acrylamide and d_3-acrylamide ensured a high degree of certainty for identification of the analyte. For acrylamide, three transitions were selected, and according to the EC criteria (*18*), this led to a total of 3 × 1.5 = 4.5 identification points. No requirements have been defined for the internal standard by the Commission Decision

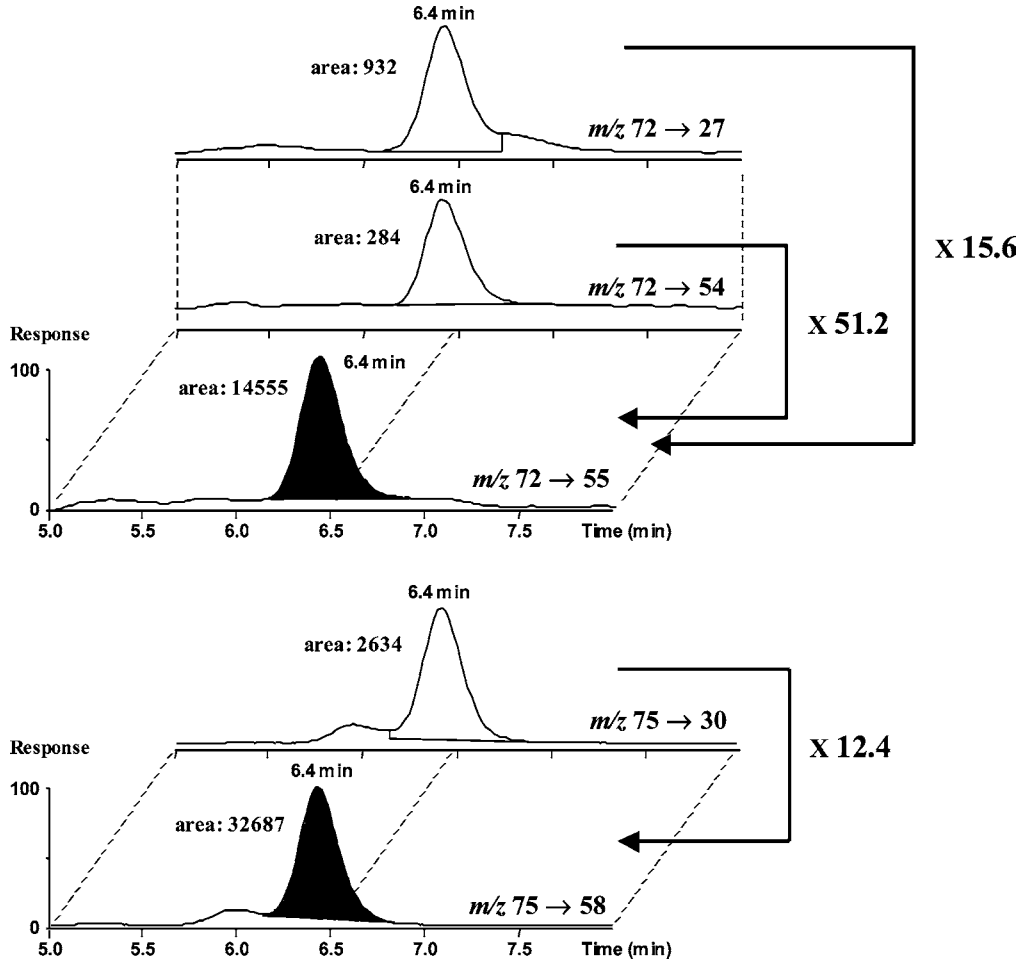

Figure 5. SRM chromatographic profile of an incurred roast and ground coffee with an acrylamide level measured at 112.5 μg/kg. Ratios between quantifiers (shaded peaks) and qualifiers are indicated. In the calibration points, the mean ratios were: *m/z* 72 → 55/*m/z* 72 → 54 = 47.2 and *m/z* 72 → 55/*m/z* 72 → 27 = 19.1.

when a tandem mass spectrometry method is employed. However, an endogenous contaminant coeluting with d_3-acrylamide could lead to an under-estimation of the level of acrylamide in foodstuffs. Therefore, two characteristic transitions were recorded for d_3-acrylamide and the relative intensities of the responses determined according to the EC requirements (*18*).

The method presented here can be applied to basically all foodstuffs known to contain traces of acrylamide. Typical SRM chromatograms are depicted in **Figures 4** and **5** for incurred soluble chocolate powder and coffee, respectively. Potential application of the method was verified by supplementing acrylamide (150 μg/kg) to roasted coffee, soluble coffee powder, coffee surrogate, and chicory samples. First, the amount of incurred acrylamide was measured in each sample. Then, the expected concentration (= incurred + supplemented concentration) of the acrylamide-fortified samples was compared. The data presented in **Table 2** show that the modified extraction procedure can be extended to various "difficult" foodstuffs.

Proficiency Tests. The method was further tested by participating in three interlaboratory collaborative trials (i.e., FAPAS/CSL) (*22*), AFSSA/LERQAP (*23*), EC/IRMM (*24*). The results are summarized in **Table 3** and show that the method compares well versus other methods ($|z| < 2$) for the quantitative determination of acrylamide in coffee, cocoa, and soluble chocolate powder. The FIRA proficiency test results are included even though our laboratory at the time this trial was conducted was employing a method that encompassed only a single ethyl acetate extraction and without the preliminary dichloromethane

Table 2. Method Suitability for Coffee-, Chocolate-, and Chicory-based Food Products

	acrylamide level (μg/kg)		
		spiked at 150μg/kg	
material	incurred[a]	expected[b]	measured[a]
roasted coffee	163	313	287
soluble coffee powder	349	499	470
coffee surrogate	673	823	802
chicory	734	884	899

[a] Mean of two independent determinations. [b] Mean incurred level + spiked level.

treatment. Under these conditions, the LOD for cocoa was estimated at 300 μg/kg, and hence acrylamide could not be quantified in the sample. This same sample was reanalyzed with the current method, obtaining 128.3 μg/kg, and suggests a lack of accuracy when compared to the interlaboratory mean at 215.2 μg/kg. However, the CV of the interlaboratory mean (34 laboratories participated in the trial) was high (214.9%) for cocoa and cannot be used as a reference value for the incurred cocoa mentioned above. Nonetheless, this highlights the importance of developing and validating methods that are suitable for the quantitative determination of acrylamide in complex food matrixes.

Similar good performance was achieved in the FIRA and EC/IRMM proficiency tests with foodstuffs such as crisp bread, butter biscuit, and butter cookie. It should be pointed out that

Table 3. Results of Acrylamide Measurements for Proficiency Tests

test, number of laboratories (*n*) and material	acrylamide level (μg/kg) interlab. mean (CV in %)	measured	*z*-score
FAPAS/CSL ($n = 31$)			
coffee	312.0 (111)	312.0	0.00
AFSSA/LERQAP ($n = 7$)			
coffee	215.0 (22.6)	208 ± 2[a]	–0.50
cocoa	282.3 (40.4)	183 ± 2[a]	–1.29
soluble chocolate powder	31.25 (47.7)	26 ± 1[a]	–1.52
EC/RMM			
crisp bread ($n = 50$)	56.9 (130)	47.0	–0.80
butter cookie ($n = 59$)	150.5 (66.0)	144.5	–0.17
spiked bread extract ($n = 60$)	116.3 (58.6)	116.0	–0.04
FIRA[b] ($n = 34$)			
cocoa	215.2 (215)	<LOD	
mashed potatoes	7286 (81.8)	8773	1.72
crisp bread 1	183.9 (242)	156.0	–0.74
crisp bread 2	181.8 (265)	152.0	–0.79
butter biscuit	531.8 (207)	469.0	–0.67

[a] Mean ± standard deviation ($n = 3$). [b] Single ethyl acetate extraction without preliminary dichloromethane-mediated cleanup. LOD in cocoa = 300 μg/kg.

Table 4. Time-Dependent Stability of Acrylamide (μg/kg) in a Soluble Chocolate Powder Extract at 93 μg/kg (mean of two independent determinations)

storage temp	initial	24 hours	48 hours	15 days	21 days
4 deg C	88.1	92.0	90.5	91.4	89.8
21 deg C	97.2	93.9	93.1	91.5	80.7

the result obtained with the acrylamide-spiked bread extract in the EC/IRMM test is particularly informative of the performance of the sample pretreatment procedure. Our measurement at 116 μg/kg represents a deviation of 0.26% of the interlaboratory mean, suggesting that no trend is generated throughout the sample extraction and cleanup.

Acrylamide Stability in Final Extracts. Information on the stability of the analyte in the final extract is part of any method validation study, as it gives information regarding the conditions under which the extracts need to be kept until further analysis. This is particularly important for acrylamide, which is sensitive to pH, light, microorganisms, and co-extractives (*12*). In this study, acrylamide in the final extracts (stored in the dark) of soluble chocolate powder appeared to be stable over a two-week period (**Table 4**), with only a slight loss (−17%) of the analyte when the extracts were stored for 21 days at 21 °C. No loss of acrylamide was observed in extracts kept refrigerated at 4 °C. However, significant change in the chromatographic profile was evident after a 15-day storage period at either 4 or 21 °C.

Acrylamide stability in food products. The stability of acrylamide was determined in selected dry food products that were reanalyzed a certain period after their initial analysis. All samples were kept in the dark at room temperature and in tightly closed containers. As shown in **Table 5**, acrylamide is stable in certain foods (breakfast cereals) over prolonged storage periods of up to 12 months. On the other hand, loss of acrylamide was appreciable in coffee (roasted and soluble) and chicory (dried and roasted) after 5−12 months storage. These observations are important as they may provide an avenue for mitigation studies (i.e., identify certain constituents in the food matrixes that may potentially "inactivate" or eliminate acrylamide).

Table 5. Time-dependent Stability of Acrylamide in Various Foodstuffs

material	interval (months)	acrylamide level (μg/kg) initial	second
breakfast cereal	12	238	238
soluble coffee powder	12	771	256
roasted barley	9	265	225
roasted coffee	7	203	147
dried chicory	5	214	174
roasted chicory	5	4015	3395
cocoa	3	180	177
chocolate with almond	2	94	73
soluble chocolate powder	1	54	41

The availability of validated robust and reliable methods to determine acrylamide at low levels in foods are of paramount importance, especially for accurate intake assessments. This is one of the first methods published to date that can be applied to all foodstuffs, addressing in particular "difficult" matrixes such as cocoa and coffee. A publication related to the determination of acrylamide in coffee appeared while the manuscript was under review (*25*). Our laboratory has conducted several hundred analyses of acrylamide on such matrixes and can with confidence propose this method as a reference method using isotope dilution LC-MS/MS.

ABBREVIATIONS USED

AFSSA/LERQAP, Agence Française de Sécurité Sanitaire des Aliments/Laboratoire d'Etudes et de Recherche en Hygiène et Qualité des Aliments; EC/IRMM, European Commission/Institute for Reference Materials and Measurements; FAPAS/CSL, Food Analysis Performance Assessment Scheme/Central Science Laboratory; FIRA, Federal Institute for Risk Assessment (Germany, BfR); JRC, Joint Research Center; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; SPE, solid-phase extraction; SRM, selected reaction monitoring.

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LITERATURE CITED

(1) Swedish National Food Administration Information about Acrylamide in Food, April 24, 2002, www.slv.se.
(2) Castle, L.; Campos, M.-J.; Gilbert, J. Determination of acrylamide monomer in hydroponically grown tomato fruits by capillary gas chromatography−mass spectrometry. *J. Sci. Food Agric.* **1991**, *54*, 549−555.
(3) Biedermann, M.; Biedermann-Brem, S.; Noti, A.; Grob, K. Two GC/MS methods for the analysis of acrylamide in foodstuff. *Mitt. Lebensm. Hyg.* **2002**, *93*, 638−652.
(4) Tateo, F.; Bononi, M. A GC/MS method for the routine determination of acrylamide in food. *Ital. J. Food Sci.* **2003**, *15*, 149−151.
(5) Ono, H.; Chuda, Y.; Ohnishi-Kameyama, M.; Yada, H.; Ishizaka, M.; Kobayashi, H.; Yoshida, M. Analysis of acrylamide by LC-MS/MS and GC-MS in processed Japanese foods. *Food Addit. Contam.* **2003**, *20*, 215−220.
(6) Nemoto, S.; Takatsuki, S.; Sasaki, K.; Maitani, T. Determination of acrylamide in foods by GC/MS using [13C1]-labeled acrylamide as the internal standard. *J. Food Hyg. Soc. Jpn.* **2002**, *43*, 371−376.
(7) Rosen, J.; Hellenas, K.-E. Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry. *Analyst* **2002**, *127*, 880−882.

(8) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Tornqvist, M. Analysis of acrylamide, a food carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.* **2002**, *50*, 4998−5006.
(9) Ahn, J. S.; Castle, L.; Clarke, D. B.; Lloyd, A. S.; Philo, M. R.; Speck, D. R. Verification of the findings of acrylamide in heated foods. *Food Addit. Contam.* **2002**, *19*, 1116−1124.
(10) Zyzak, D. V.; Sanders, R. A.; Stojanovich, M.; Tallmadge, D. H.; Eberhart, B. L.; Ewald, D. K.; Gruber, D. C.; Morsch, T. R.; Strothers, M. A.; Rizzi, G. P.; Villagran, M. D. Acrylamide formation in heated foods. *J. Agric. Food Chem.* **2003**, *51*, 4782−4787.
(11) Becalski, A.; Lau, B. P. Y.; Lewis, D.; Seaman, S. W. Acrylamide in foods: occurrence, sources, and modeling. *J. Agric. Food Chem.* **2003**, *51*, 802−808.
(12) Roach, J. A. G.; Andrzejewski, D.; Gay, M. L.; Nortrup, D.; Musser, S. M. Rugged LC-MS/MS survey analysis for acrylamide in foods. *J. Agric. Food Chem.* **2003**, *51*, 7547−7554.
(13) Jezussek, M.; Schieberle, P. A new LC/MS-method for the quantitation of acrylamide based on a stable isotope dilution assay and derivatization with 2-mercaptobenzoic acid. Comparison with two GC/MS methods. *J. Agric. Food Chem.* **2003**, *51*, 7866−7871.
(14) Riediker, S., Stadler, R. H. Analysis of acrylamide in food by isotope-dilution liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *J. Chromatogr. A.* **2003**, *1020*, 121−130.
(15) Wenzl, T.; de la Calle, B.; Anklam, E. Analytical methods for the determination of acrylamide in food products: a review. *Food Addit. Contam.* **2003**, *20*, 885−902.
(16) Interlaboratory trial of the Federal Institute for Risk Assessment, **2002**, Germany, October, Fauhl, C.; Klaffke, H.; Mathar, W.; Palavinskas, R.; Wittkowski, R.
(17) Report of the European Workshop on Analytical Methods for the Determination of Acrylamide in Food Products, jointly organised by EC JRC & DG SANCO, Oud-Turnhout, Belgium, **2003**, 28−29 April.
(18) Commission Decision 2002/657/EC, Aug 12, 2002, Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Off. J. Eur. Communities* **2002**, *L221*, 8−36.
(19) Polzer, J.; Gowik, P. Validation of a method for the detection and confirmation of nitroimidazoles and corresponding hydroxy metabolites in turkey and swine muscle by means of gas chromatography-negative ion chemical ionization mass spectrometry. *J. Chromatogr. B* **2001**, *761*, 47−60.
(20) Jülicher, B.; Gowik, P.; Uhlig, S. Assessment of detection methods in trace analysis by means of a statistically based in-house validation concept. *Analyst* **1998**, *123*, 173−179.
(21) Mottier, P.; Parisod, V.; Gremaud, E.; Guy, P. A.; Stadler, R. H. Determination of the antibiotic chloramphenicol in meat and seafood products by liquid chromatography-electrospray ionization tandem mass spectrometry. *J. Chromatogr. A* **2003**, *994*, 75−84.
(22) Interlaboratory trial of the Food Analysis Performance Assessment Scheme, Central Science Laboratory, UK, 2003, September.
(23) Interlaboratory trial of the Agence Française de Sécurité Sanitaire des Aliments, Laboratoire d'Etudes et de Recherche en Hygiène et Qualité des Aliments, France, 2003, February.
(24) Interlaboratory trial of the European Commission, Joint Research Center/Institute for Reference Materials and Measurements, Belgium, 2003, September, Wenzl, T.; de la Calle, B.; Ulberth, F.; Anklam, E.
(25) Andrzejewski, D.; Roach, J. A. G.; Gay, M. L.; Musser, S. M. Analysis of coffee for the presence of acrylamide by LC-MS/MS. *J. Agric. Food Chem.* **2004**, *52*, 1996−2002.

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