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Determination of acrylamide and methacrylamide by normal phase high performance liquid chromatography and UV detection

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Abstract

A method using normal phase high performance liquid chromatography (NP-HPLC) with UV detection was developed for the analysis of acrylamide and methacrylamide. The method relies on the chromatographic separation of these analytes on a polar HPLC column designed for the separation of organic acids. Identification of acrylamide and methacrylamide is approached dually, that is directly in their protonated forms and as their hydrolysis products acrylic and methacrylic acid respectively, for confirmation. Detection and quantification is performed at 200 nm. The method is simple allowing for clear resolution of the target peaks from any interfering substances. Detection limits of 10 $\mu g\,L^{-1}$ were obtained for both analytes with the inter- and intra-day RSD for standard analysis lying below 1.0%. Use of acetonitrile in the elution solvent lowers detection limits and retention times, without impairing resolution of peaks. The method was applied for the determination of acrylamide and methacrylamide in spiked food samples without native acrylamide yielding recoveries between 95 and 103%. Finally, commercial samples of french and roasted fries, cookies, cocoa and coffee were analyzed to assess applicability of the method towards acrylamide, giving results similar with those reported in the literature.

Keywords: Acrylamide; Methacrylamide; Acrylic acid; Methacrylic acid; Normal phase HPLC; UV detection

1. Introduction

Acrylamide (2-propenamide) is a low molecular weight hydrophilic compound known mostly for its use as a monomer in the production of polyacrylamide, which in turn is used in plastics and as an electrophoresis medium [1–3]. Although it is classified as a probable human carcinogen and it has been incriminated for mutagenicity in rats and as a human neurotoxin, its presence in food has received little attention as no or negligible migration has been reported from the plastic packaging material [3–5]. The whole picture was changed after the announcement made in 2002 by the Swedish National Food Administration [6], which reported extremely high levels of acrylamide in products that are consumed on a regular basis in rather large quantities such as potato crisps, roast potatoes, breakfast cereals and bakery products. Since then, several papers were published trying to postulate the mechanism via which acrylamide is formed [7–10]. The conclusion to date was that acrylamide is formed during cooking, frying and baking at temperatures exceeding 120 °C of fatty, carbohydrate and asparagine-rich foods either as product of asparagine-glucose reaction (Maillard reaction) [8–10] or through decomposition of triglycerides.

An urgent requirement is the development and validation of sensitive, robust and inexpensive analytical methods that can quantify acrylamide in different food matrices down to the low μg/kg level [6,10,11]. Numerous methods have in fact been developed in the past years to determine the acrylamide monomer, especially in water, biological fluids and food. The majority of them are based on liquid or gas chromatographic techniques [12–27]. However, these methods as such, do not suffice for the analysis of acrylamide in processed/cooked foods at low levels. In particular they lack selectivity and the additional degree of analyte certainty required to confirm the presence of a small molecule such as acrylamide in a complex food matrix.

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To date, several analytical methods dealing with the analysis of acrylamide in cooked foods have been published. These methods are based mainly on mass spectrometry (MS) as the determinative technique, coupled with a chromatographic step either by LC [18–20] or GC [21–25], usually after derivatization of the analyte. In fact, the expert Working Group on Analytical Methods that convened during the recent JIFSAN meeting on acrylamide [10] concluded, that the majority of laboratories use either GC–MS or LC–MS, the advantage of the LC–MS-based methods being that acrylamide can be analyzed without prior derivatization (e.g. bromination), which considerably simplifies and expedites the analysis. Due to the low molecular mass of acrylamide (71 g/mol) and thus also its low-mass fragment ions, confirmation of the analyte can be achieved with a two-stage mass spectrometer (monitoring of more than one characteristic mass transition) [18–24]. However, acrylamide is a very polar molecule with poor retention on conventional LC reversed-phase sorbents [22], and despite the use of tandem mass spectrometry more effort may need to be placed on efficient clean-up steps to avoid interference from co-extractives.

Normal Phase chromatographic separation has been neglected although there are some papers that encourage such an approach [26,27]. Especially in the work supported by Dionex [26], ion chromatography with UV detection at 202 nm was used successfully for the determination of acrylamide provided that a thorough clean-up step had preceded analysis. The obtained results were found similar with those obtained with MS detection even at 0.1 mg/kg level. Therefore, UV detection can be used for the determination of reported concentrations of acrylamide, which for the inspected food products range from 0.040 to 0.050 mg/kg for coffee and pastry, up to 2 and 3 mg/kg for fried potatotes. These detection limits can be further reduced by appropriate clean-up.

In the present work, we propose the use of NP-HPLC as an effective method for detection and quantification of acrylamide. The same methodology is also applicable for the determination of its methylated product methacrylamide, although there are no real sample applications. The method is based on the separation of these analytes on a polar HPLC column (Aminex HPX-87H) using 0.01 M sulfuric acid as eluent. Under these conditions both analytes are converted into their cationic, protonated, ammonium products. The peaks of acrylamide and methacrylamide are well resolved not only one from the other but from other compounds as well, since both analytes appear far later than any other in the chromatogram. In order to confirm our results avoiding MS, both analytes were hydrolyzed and converted to their respective acids and determined as such. Resolution of the acid peaks (acrylic and methacrylic) revealed the possibility of acrylamide and methacrylamide being analyzed in these forms. Enhancement in performance and reduction in analysis times was further achieved by introduction of a 20% acetonitrile gradient in the mobile phase.

2. Experimental

2.1. Apparatus and software

The liquid chromatograph consisted of a Shimadzu 10AD series for HPLC equipped with a UV–vis variable wavelength detector (Shimadzu) set at 200 nm. An Aminex HPX-87H (300 mm × 7.8 mm) column for organic acid analysis supplied by Bio-Rad and thermostated at 30 °C in a CTO-10A Shimadzu column oven, was used for all separations. Data collection and manipulation was performed by means of a CLASS-VP Shimadzu automated software for chromatography. A Vortex Velp Scientifica mixer was used for thorough mixing of solutions. A Sorvall RC-5B Refrigerated Superspeed Centrifuge (Du Pont Instruments) was used for centifugal separations. A thermostated bath maintained at the desired temperatures was used for heating experiments.

2.2. Reagents

All reagents were of analytical grade or of the highest grade available. Acrylamide and methacrylamide along with their respective acids were obtained from Sigma Aldrich Chemical Company (USA). Suitable amounts of each compound were dissolved in 100 ml of distilled water to prepare 1 mg L^{-1} stock solutions. Working solutions of each compound were prepared daily by appropriate dilutions of the standard solutions. Water and Acetonitrile (Merck, Darmstadt Germany) used for chromatographic separation were HPLC grade.

2.3. Samples

All samples (raw chicken, potatoes, chocolate chip cookies, cocoa and Greek coffee) were purchased from a local super-market. Potatoes were sliced in round pieces and either baked in an oven at 250 °C for 1 h or fried in olive oil (T = 180 °C) for ca. 25 min.

2.4. General procedure

2.4.1. Sample preparation

The extraction of acrylamide from real samples was performed with water as the extractant, according to a combination of the optimum conditions cited in the literature [25]. Five grams of each food sample were ground in a Waring blender for 3 min and thoroughly homogenized with 5 mL of distilled water. Another 10 mL of water were added and the homogenates were left to stand in a thermostated water bath set at 70 °C under agitation. After 30 min the homogenates were centrifuged (12000 rpm, 20 min, 4 °C) to allow precipitation and filtered twice through Whatman No. 2 filter paper. The filtrates were transferred to 10 mL volumetric flasks and diluted with distilled water until mark. Before analysis hexane (three portion of 3 mL) were added to 1 mL of the sam-

ple solution to extract remaining long chain fatty acids that could create problems in chromatographic analysis by giving peaks overlapping with the target analytes or block the polar column. The mixture was shaken vigorously and the upper hexane layer was removed with a Pasteur pipette. 20 μL were taken with a Hamilton syringe directly from the lower aqueous layer and injected into the HPLC to be analyzed for acrylamide and methacrylamide.

Alternatively after centrifugation 5 ml of a 4 M NaOH solution was added to the supernatant which was then heated to 70 °C for 1 h in order for the amides to be converted to the respective carboxylate derivatives and the final solution was again made up to 10 mL with a 2 M phosphate buffer solution (pH 2). Lipophilic remains were again extracted with hexane and 20 μL of the aqueous layer were injected in the HPLC column for acrylamide and methacrylamide to be determined as their derivative acids.

2.5. Chromatographic conditions for the separation of acrylamide, methacrylamide, acrylic and methacrylic acid

An isocratic elution pattern was adopted for the separation of the target analytes. 0.01 M sulfuric acid solution was used throughout the analysis. Alternatively a 20–80% acetonitrile–0.01 M sulfuric acid mixture was used to shorten the analysis time and enhance detectability. In all cases the column temperature was set at 30 °C, flow rate was maintained at 0.6 mL min^{-1} while detection was performed at 200 nm.

3. Results and discussion

3.1. Direct NP-HPLC determination of acrylamide and methacrylamide

The first aim of this work was to determine the optimum conditions for obtaining maximum and well resolved peaks. Based on the fact that the concept of the method was approached on the acid–base interaction of the target analytes with the polar column, that is, that both acrylamide and methacrylamide would be separated via their pK_a (protonated) or pK_b (amide) values, aqueous acidic solutions were tested in order to decide upon the optimum eluent. Among the common inorganic acids tested (HNO_3, HCl, $HClO_4$ and H_2SO_4) sulfuric acid—also recommended for the separation of organic acids—gave the optimum results. A 0.01 M sulfuric acid solution gave the highest peaks and although more acidic eluents (Fig. 1a) resulted in shorter retention times by 1 to 3 min the 0.01 M solution was finally selected in order to obtain the lowest detection limits possible.

The effect of flow rate values was also evaluated within the range 0.1–1.0 mL min^{-1}. It was found that 0.6 mL min^{-1} was the optimum value because although greater flow rates reduced the run time by almost 10 min for the amides, they impaired the resolution while reducing the obtained peak area values (Fig. 1b). Flow rates exceeding 1.0 mL min^{-1} resulted in poor elution and double peaks of the target analytes.

Column temperature variation was also evaluated given that increased temperatures result in shorter retention times. Indeed retention times were slightly reduced but with a cost in signal intensity (Fig. 1c). Therefore the optimum temperature was selected to be 30 °C.

Under the above conditions calibration curves were constructed for both acrylamide and methacrylamide. As can be seen from Table 1 both analytes can be detected and quantified at the μg L^{-1} level and therefore the proposed method can be easily applied to the target products described in the introduction.

3.2. NP-HPLC determination of acrylamide and methacrylamide as their hydrolysis products: acrylic and methacrylic acid

It is common knowledge that amides can be transformed into their precursor carboxylic acids (or carboxylate anions) by acid or base catalyzed hydrolysis. Since the HPLC column used is recommended for the separation of these acids an attempt was made to convert acrylamide and methacrylamide into acrylic and methacrylic acid and consequently determine them as such. Heating (70 °C) of both analytes for 6 h in 5 M sulfuric acid or for 1 h in 4 M NaOH resulted in complete disappearance of the amide peaks while two new peaks corresponding to the respective acids emerged. It came as no surprise that these peaks had retention times 18–21 min shorter than those of the amides (Fig. 2) as the acids have pK_a values by several orders of magnitude greater than the protonated amides.

Under the aforementioned optimized conditions for the amides, their precursor acids gave identical calibration curves (Table 1) while the overall gain of this approach was not only a substantial reduction in analysis time and solvents waste but—most important—a verification of the amide findings. That is, after the direct analysis approach, the sample could be hydrolyzed and reanalyzed for acrylic and methacrylic acid for confirmation.

After all, a major objective of this work was to confirm that a NP-HPLC method could separate the target analytes effectively and reproducibly. A first approach was to check the intra and inter day reproducibility of the proposed method by comparing the retention times and areas of the obtained peaks of a standard containing 200 μg L^{-1} of each compound. After 10 consecutive injections and over a period of 1 week, it was proven that the obtained retention times (Table 1), had an RSD below 1.0% as they varied in the second decimal digit. There was therefore by no means confirmation of the early assumption that HPLC cannot be accounted for reliable determination of acrylamide by retention times.

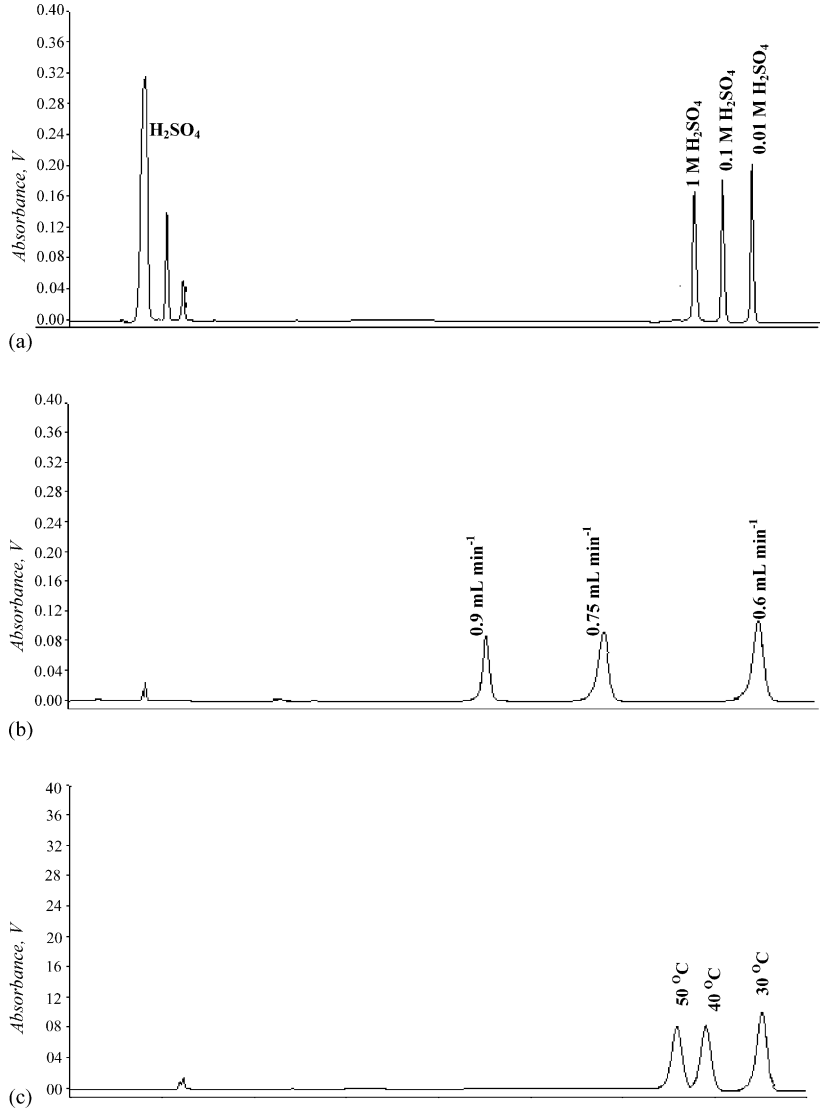

Fig. 1. Effect of: (a) eluent acidity, (b) elution rate and (c) column temperature, on the peak profile of acrylamide after NP-HPLC separation.

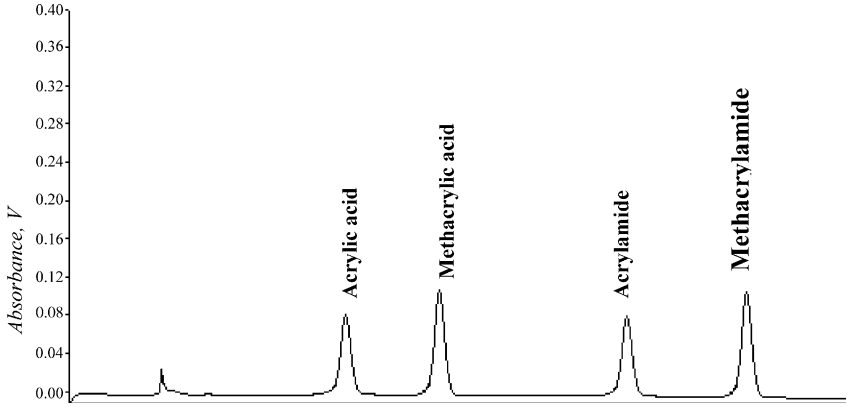

Fig. 2. Superimposed chromatograms comparing the peaks obtained for acrylamide and methacrylamide (0.2 mg L^{-1}) and for acrylic and methacrylic acid after hydrolysis of the respective amides.

Table 1
Analytical characteristics of the proposed methodologies for the determination of acrylamide and methacrylamide

	100% Sulfuric acid 0.01 M				80–20% Sulfuric acid 0.01 M–acetonitrile			
	Acrylamide	Methacrylamide	Acrylic	Methacrylic	Acrylamide	Methacrylamide	Acrylic	Methacrylic
Retention time	36.25 ± 0.08	44.18 ± 0.09	18.15 ± 0.06	23.91 ± 0.07	27.51 ± 0.08	34.65 ± 0.08	13.54 ± 0.05	19.23 ± 0.04
Intra-day %RSD	0.4	0.4	0.6	0.6	0.4	0.4	0.6	0.5
Inter-day %RSD	0.5	0.5	0.7	0.7	0.5	0.5	0.7	0.6
Regression equation	$S^{a} = 301C^{b} + 1630$	$S = 303C + 5621$	$S = 305C + 1570$	$S = 308C + 5543$	$S = 390C + 6351$	$S = 391C + 2183$	$S = 394C + 5987$	$S = 399C + 1896$
LOD[c]	15	12	15	12	1.5	1.0	1.5	1.0
LOQ[d]	45	35	45	35	4.0	3.0	4.0	3.0
Correlation coefficient (R^2)	0.9998	0.9998	0.9999	0.9999	1	1	1	1
Linear range[e]	45–2000	35–2000	45–2000	35–2000	4.0–2000	3.0–2000	4.0–2000	3.0–2000

[a] Peak area (arbitrary units).
[b] Concentration ($\mu g\,L^{-1}$).
[c] Limit of detection defined as three times the signal to noise ratio ($\mu g\,L^{-1}$).
[d] Limit of quantitation defined as 10 times the signal to noise ratio ($\mu g\,L^{-1}$).
[e] $\mu g\,L^{-1}$.

Drawbacks from this approach may stem from the fact that acrylic acid and 15 of its esters are monomers used to make polymers, intended to come into contact with foods. Therefore these esters may migrate into foods generating free acrylic acid. Although this fact may seem as major source of interference this is not the case with the proposed methodology because acrylamide can be differentiated even in this case since acrylic acid would be quantified in the first run for acrylamide, while the acrylamide presence could be verified after hydrolysis by an increase in acrylic acid's peak.

3.3. Effect of acetonitrile on the chromatographic separation and determination of acrylamide/acrylic acid and methacrylamide/methacrylic acid

In a further optimization approach, other HPLC solvents were used in order to examine the possibility of resolution and signal enhancement along with a further reduction in the duration of the chromatographic run.

The detection wavelength (200 nm) is a limiting factor as most of the HPLC solvents have a wavelength cut-off well above it. We found that introduction of acetonitrile not only enhanced the signal but also decreased the obtained retention times suppressing those of the organic acids to the beginning of the chromatogram. Optimization of the acetonitrile gradient with a view of better peaks and shorter retention times revealed an optimum performance for a 20% acetonitrile content. As can be seen from Table 1 the detection and quantification limits are lowered to 0.5 and $2\,\mu g\,L^{-1}$ respectively while the use of acetonitrile improved the linearity of the obtained calibration curves yielding correlation coefficient (R^2) 0.9999–1 which is probably the reason for the improved performance.

3.4. Recovery experiments and analysis of spiked and commercial food samples

Having completed three sets of experimental conditions mainly for the determination of both acrylamide and methacrylamide, validation of their performance was approached by spiking a sample of raw chicken meat with the target analytes and performing recovery tests with all three proposed methods. More specifically, the extract obtained after the procedure, described under sample preparation, was spiked with known amounts of acrylamide and methacrylamide concentrated stock solution and submitted to analysis following all the proposed approaches. The results, depicted in Tables 2 and 3, show that recoveries in the range 95–105% that approach 100% in the low concentration region recommend NP-HPLC to be valid for sensitive acrylamide and methacrylamide determination, while the agreement between the amide and respective acid finding suggest that this approach may offer an inexpensive alternative to

Table 2
Recovery sudies of acrylamide and methacrylamide after spiking a raw chicken sample

Added ($\mu g\,kg^{-1}$)	Found as			
	Acrylamide ($\mu g\,kg^{-1}$)	Recovery (%)	Acrylic acid ($\mu g\,kg^{-1}$)	Recovery (%)
Eluent: 100% sulfuric acid 0.01 M				
Acrylamide				
100	100	100.0	101	101.0
200	199	99.5	200	100.0
1000	980	98.0	990	99.0
2000	1900	95.0	1940	97.0
Eluent: 80–20% sulfuric acid 0.01 M–acetonitrile				
10	9.9	99.0	10.0	100.0
50	49.2	98.4	51.0	102.0
200	194	97.0	198	99.0
500	490	98.0	484	96.8
1000	990	99.0	980	98.0
	Methacrylamide ($\mu g\,kg^{-1}$)	Recovery (%)	Methacrylic acid ($\mu g\,kg^{-1}$)	Recovery (%)
Eluent: 100% sulfuric acid 0.01 M				
Methacrylamide				
100	102	102.0	105	105.0
200	195	97.5	204	102.0
1000	9990	99.9	1000	100.0
2000	1940	97.0	1990	99.5
Eluent: 80–0% sulfuric acid 0.01 M–acetonitrile				
10	10.0	100.0	10.1	101.0
50	51.0	102.0	52.0	104.0
200	190	95.0	201	100.5
500	500	100.0	498	99.6
1000	940	94.0	980	98.0

the use of mass selective detection. The extraction procedure applied along with the de-fatting of the extract gave clear chromatograms with no interfering peaks appearing (Fig. 3) at the desired retention times. The concentrations of acrylamide determined in all the analyzed samples are in good agreement with the average values reported in the literature while methacrylamide was not detected in any of the samples.

Table 3
Analysis of acrylamide in food samples

Sample	Concentration ($\mu g\,kg^{-1}$)	Added ($\mu g\,kg^{-1}$)	Determined[a] ($\mu g\,kg^{-1}$)	Recovery (%)
Acrylamide				
Fried potatoes	843.3 ± 0.9	–	–	–
		10	853.8 ± 0.7	105.0
		50	894.5 ± 0.9	102.4
		100	942.9 ± 0.9	99.6
Baked potatoes	238.2 ± 0.5	–	–	–
		10	248.5	103.0
		50	289.3	102.2
Chocolate cookies	400.4 ± 0.7	–	–	–
		50	451.2	101.4
		100	499.8	99.4
Cocoa	83.8 ± 0.4	–	–	–
		10	93.1 ± 0.3	93.0
		20	103.0	96.0
Coffee	68.0			
		10	77.5	95.0
		20	87.1	95.5

[a] Average of three experiments.

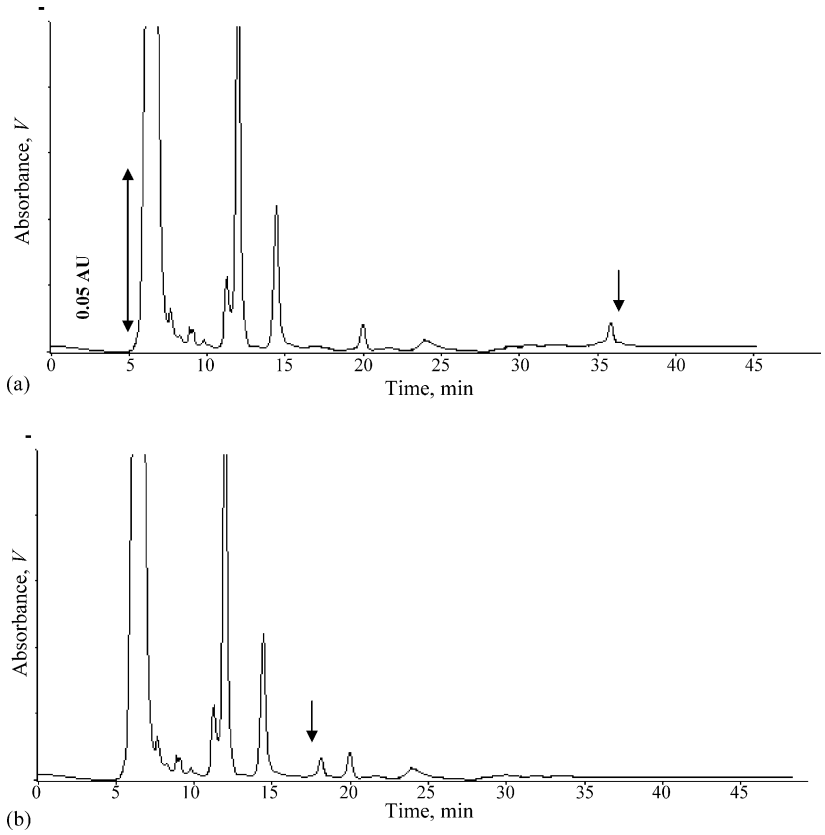

Fig. 3. Chromatogram obtained from the analysis of a chocolate chip cookie sample extract: (a) directly for acrylamide and (b) after hydrolysis. Arrow pointed peaks are acrylamide and acrylic acid respectively. Remaining peaks correspond to organic acids. Chromatographic conditions as reported in the text.

4. Conclusions

Two approaches are proposed for the determination of acrylamide and methacrylamide with NP-HPLC with acrylamide being the main target due to its aledged genotoxicity and increased occurrence in high consumption foods. Detection limits in the low $\mu g\,L^{-1}$ level along with increased linearity and high precision suggest that these can be applied as cost effective alternatives of the MS methods applied so far without the implication of time consuming and hazardous bromination step. Further enhancement of the signal and confinement of the chromatographic run to ca. 15 min allows for rapid and reliable analysis of acrylamide. Finaly, although methacrylamide was not determined in any real sample its alike behavior can find potential application in extraction experiments since its absence from real samples enables its use as an internal standard.

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References

[1] K.L. Dearfield, C. Abernathy, M. Ottley, J. Brantner, P. Hayes, Mutat. Res. 195 (1988) 45.
[2] EEC, Commission Directive 2002/72/EEC. Off. J. Eur. Com. L220 (2002) 18 (and amendment L039 (2003) 1).
[3] E.A. Smith, F.W. Oehme, Rev. Environ. Health 9 (1991) 215.
[4] K.L. Dearfield, G.R. Douglas, U.H. Ehling, M.M. Moore, G.A. Sega, D.J. Brusick, Mutat. Res. 330 (1995) 71.
[5] IARC Monographs in the Evaluation of Carcinogenic Risks to Humans, vol. 60, International Agency for Research on Cancer, Lyon, 1994, p. 389.
[6] Swedish National Food Administration, 2002, (http://www.slv.se/).
[7] C. Gertz, S. Klostermann, Eur. J. Lipid Sci. Technol. 104 (2002) 762.
[8] V.A. Yaylayan, A. Wnorowski, C. Perez-Locas, J. Agric. Food Chem. 51 (2003) 1753.
[9] D.S. Mottram, B.L. Wedzicha, A.T. Dodson, Nature 419 (2002) 448.
[10] R.H. Stadler, I. Blank, N. Varga, F. Robert, J. Hau, P.A. Guy, M.-C. Robert, S. Riediker, Nature 419 (2002) 449, Report of the Analytical Working roup, 9 November 2002, JIFSAN Acrylamide in Food Workshop, 28–30 October, Chicago, USA, http://www.jifsan.umd.edu/acrylamide2004.htm (and references mentioned therein).
[11] E. Dybing, P.B. Farmer, M. Andersen, T.R. Fennell, S.P.D. Lalljie, D.J.G. Müller, S. Olin, B.J. Petersen, J. Schlatter, G. Scholz, J.A. Scimeca, N. Slimani, M. Törnqvist, S. Tuijtelaars, P. Verger, Food Chem. Toxicol. 43 (2005) 365.
[12] L. Castle, J. Agric. Food Chem. 41 (1993) 1261.

[13] US EPA, SW 846, Method 8032A, US Environmental Protection Agency, Washington, DC, 1996.
[14] L.S. Bologna, F.F. Andrawes, F.W. Barvenik, R.D. Lentz, R.E.J. Sojka, J. Chromatogr. Sci. 37 (1999) 240.
[15] D.S. Barber, J. Hunt, R.M. LoPachin, M. Ehrich, J. Chromatogr. B 758 (2001) 289.
[16] E. Tareke, P. Rydberg, P. Karlsson, S. Eriksson, M. Törnqvist, Chem. Res. Toxicol. 13 (2000) 517.
[17] K. Kawata, T. Ibaraki, A. Tanabe, H. Yagoh, A. Shinoda, H. Susuki, A. Yasuhara, J. Chromatogr. A 911 (1) (2001) 75.
[18] J. Rosén, K.-E. Hellenäs, Analyst 127 (2002) 880.
[19] E. Tareke, P. Rydberg, P. Karlsson, S. Eriksson, M. Törnqvist, J. Agric. Food Chem. 50 (2002) 4998.
[20] A. Becalski, B.P.-Y. Lau, D. Lewis, S.W. Seaman, J. Agric. Food Chem. 51 (2003) 802.
[21] M. Biedermann, S. Biedermann-Brem, A. Noti, K. Grob, Mitt. Lebensm. Hyg. 93 (2002) 638.
[22] J.S. Ahn, L. Castle, D.B. Clarke, A.S. Lloyd, M.R. Phillo, D.R. Speck, Food Addit. Contam. 19 (2002) 1116.
[23] F. Andrawes, S. Greenhouse, D. Draney, J. Chromatogr. 399 (1987) 269.
[24] H. Ono, Y. Chuda, M. Ohnishi-Kameyama, H. Yada, M. Ishizaki, H. Kobayashi, M. Yoshida, Food Addit. Contam. 20 (2003) 215.
[25] T. Wenzl, B.M. de la Calle, E. Anklam, Food Addit. Contam. 20 (2003) 885 (along with included references).
[26] S. Cavalli, R. Maurer, F. Höfler, LC–GC Eur., The Applications Book, 2003, p. 1.
[27] F. Höfler, R. Maurer, S. Cavalli, GIT Labor-Fachzeitshrift 9 (2002) 968.