

Rapid, Sensitive and Selective Analysis of Acrylamide in Cereal Products Using Bromination and GC/MS/MS

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Abstract: A rapid and sensitive method has been developed and validated for the analysis of acrylamide in cereal products. A kinetic study showed that a quantitative bromination of acrylamide in cereal extracts could be achieved within 25 min at room temperature. By using GC/MS/MS an increase in signal to noise of between 70 to 100 could be achieved for samples with minimal clean up compared to conventional selected ion monitoring. Results obtained from the analysis of a FAPAS cereal test material showed good agreement with the assigned value.

Keywords: acrylamide; bromination; kinetics; GC/MS/MS; tandem mass spectrometry

INTRODUCTION

Since the emergence of acrylamide as an issue in foods methods based on GC/MS of the brominated derivative [1] and LC/MS of free acrylamide [2] have found widespread use. Expert working groups have identified the requirement for sensitive and robust methods for the analysis of acrylamide in different matrices as a priority [3].

The division between methods based on GC/MS and LC/MS is roughly equal [4]. The analysis of free acrylamide by LC/MS requires tandem mass spectrometry (MS/MS) and/or exhaustive clean-up to achieve selective detection at low molecular weight. For GC/MS methods, this objective is achieved *via* bromination of acrylamide to give 2,3-dibromopropionamide (2,3-DBP). The addition of molecular bromine to acrylamide occurs via an ionic mechanism and the conditions of bromination vary widely: bromine can be generated *in situ* [5] or added as an aqueous reagent [6]. Reaction times of 1–15 h have been used [5–8] and, to date, no data on the kinetics of bromination in a food matrix have been reported. Under some GC conditions, it has been shown that 2,3-DCP is unstable and can be converted (via dehydrobromination) to the more stable 2-bromopropenamide (2-BP) [9].

Detection limits of less than 50 µg/kg can not be achieved in many cereal products without ad-

ditional and time consuming sample preparation procedures [10]. This limitation could be overcome by using MS/MS to monitor specific mass transitions from 2,3-DBP. The objectives of this study were twofold: a) to study the kinetics of acrylamide bromination in a cereal matrix and b) to develop and apply MS/MS for selective detection with minimal sample clean-up.

EXPERIMENTAL

Cereal samples. FAPAS test materials (series 30) were purchased from the Central Science Laboratory (York, UK). Retail sliced white bread was air-dried and homogenised. Bread with acrylamide added pre-bake (via dough water at equivalent of 1000 µg/kg on flour weight) was prepared by the Chorleywood baking process [11], freeze-dried and homogenised.

Chemicals. Acrylamide (99%), bromine (99.99%), hydrobromic acid (48%), glacial acetic acid, potassium hexacyanoferrate(II) trihydrate, sodium sulphate anhydrous, sodium thiosulphate pentahydrate, triethylamine (99.5%) and zinc sulphate heptahydrate were from Aldrich (Gillingham, UK). Potassium bromide, BDH AnalaR® was from VWR International Ltd (Poole, UK). Acrylamide-1,2,3-¹³C₃ was from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Deionised water was pre-

pared in-house (NANOpure® DIamond™, Thame, UK). Sodium sulphate and potassium bromide were purified at 600°C overnight (muffle furnace).

Standards and reagents. Stock solutions of acrylamide and $^{13}\text{C}_3$ -acrylamide (ISTD) were prepared at 1 mg/ml in deionised water. An ISTD spiking solution was diluted to 25 µg/ml; acrylamide calibration standards were prepared in the range 0–1000 µg/l each with an ISTD concentration of 250 µg/l. Brominating reagent (stored at 3°C) was prepared according to the procedure of CASTLE [6]. Carrez I and II were prepared at 85mM and 250mM, respectively.

Sample extraction. Homogeneous samples (5 g) were weighed into 50 ml centrifuge tubes and spiked with ISTD (50 µl of 25 µg/ml). Deionised water was added and the sample macerated for 1 min with a Ystral 10T shaft (Sweden). The homogenate was acidified with glacial acetic acid (~0.3 ml), treated successively with Carrez I and II (1 ml of each) and centrifuged at 5°C until a clear layer formed (1942 g_{av} 20 min).

Bromination. The clear aqueous layer (10 ml) or calibration standard (2 ml) was treated with 15 ml of chilled brominating reagent (3 ml for calibration standards) in a centrifuge tube. The covered tube (tin foil) was allowed to stand at room temperature for 30 min. The reaction was stopped and excess bromine decomposed by the addition of 2–3 drops of sodium thiosulphate. The clear solution was then shaken with 5 ml ethyl acetate (2 ml for calibration standards) for 1 min, centrifuged (1942 g_{av} for 5 min) to aid phase separation and the organic layer dried over anhydrous sodium sulphate. Triethylamine (50 µl) was added to convert 2,3-DBP to 2-BP.

Bromination kinetics. Aqueous breadcrumb extract (1:10 crumb/water w/v) was spiked with acrylamide at 100 µg/ml. Spiked extract (2 ml) was treated with brominating reagent (3 ml) at either 0°C or 21°C. The reaction was stopped by the addition of sodium thiosulphate and the product extracted into ethyl acetate (10 ml). Samples were analysed by GC-FID using on-column injection onto a 30 m × 0.25 mm i.d. Rtx®-50 (Restek, Bellefonte, PA) column with a 0.25 µm film thickness. The GC oven was programmed from 65°C (2 min hold) to 250°C at 15°C/min (5 min hold).

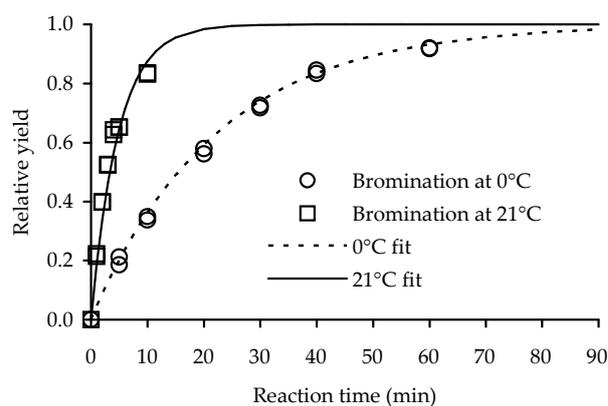
GC/MS/MS. Sample extracts and calibration standards were analysed using a Varian 1200L GC/MS/MS system (Walnut Creek, CA, USA). Splitless injections (1 µl) were made using the GC column

and conditions described above. Argon collision gas (1.5 mTorr) at a collision energy of –10eV was used and the following parent-to-daughter transitions (MRM) were monitored (boldface type denotes transition used for quantification): m/z 149 > 70 and m/z 151 > 70 (acrylamide) and m/z 152 > 73 and m/z 154 > 73 ($^{13}\text{C}_3$ -acrylamide). Acrylamide was quantified by a stable isotope internal standard method using the ratio of the m/z 149 > 70/154 > 73 MRM responses and the slope of a least squares fit to a seven point calibration data set.

RESULTS AND DISCUSSION

Bromination kinetics. The kinetics of acrylamide bromination in a sample matrix was assessed using spiked aqueous breadcrumb extract (100 µg/ml acrylamide) and GC-FID. The maximum yield of 2,3-DBP was attained within 90 min at 0°C and 25 min at 21°C (Figure 1). The experimentally determined second order rate constants were $2.844 \times 10^{-6} \text{ m}^3/\text{mol}\cdot\text{s}$ and $1.605 \times 10^{-5} \text{ m}^3/\text{mol}\cdot\text{s}$ at 0°C and 21°C, respectively (in excess bromine). The recovery of 2,3-DBP was similar at each temperature indicating that possible side reactions at elevated temperature were negligible. The overall recovery of 2,3-DBP in the ethyl acetate extract was approximately 80% based on the FID response of non-derivatised acrylamide reference standard.

Stability. Using on-column injection, standard solutions of 2,3-DBP could be analysed without conversion to 2-BP. However, chromatographic performance deteriorated with sample extracts



For the reaction: $A + B \xrightarrow{k} AB$

where: A = acrylamide; B = Br_2 (in excess); AB = 2,3-DBP;
k = second order rate constant

Figure 1. Kinetics of acrylamide bromination in breadcrumb extract at 0°C and 21°C

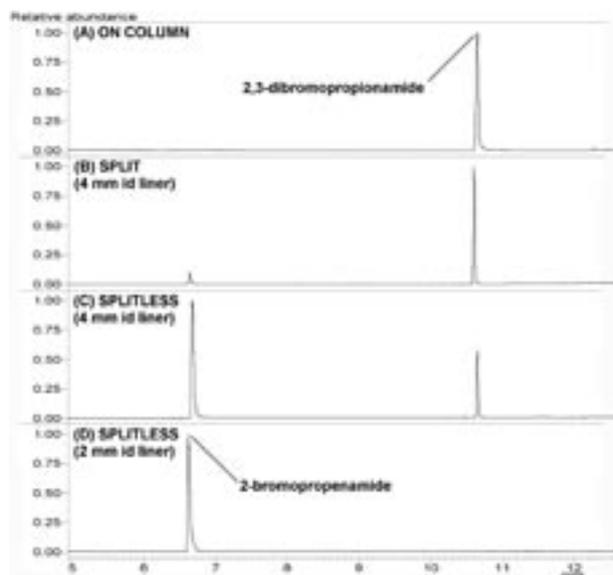


Figure 2. Effect of injection technique on the stability of 2,3-DBP

due to the build up of co-extracted material on the front of the GC column. When 2,3-DBP was introduced to the GC using split/splitless injection conversion to 2-BP was dependent upon residence time in the injector and the liner volume (Figure 2). Hence, 2,3-DBP was deliberately converted to 2-bromopropenamide prior to injection [9] to ensure reliable quantification.

GC/MS/MS. Parent ions at m/z 149 [$C_3H_4^{79}BrNO$]⁺ and m/z 151 [$C_3H_4^{81}BrNO$]⁺ from the EI mass spectrum of acrylamide [6] were chosen for identification and quantification. The corresponding parent ions, i.e. m/z 152/154 were used for $^{13}C_3$ -acrylamide

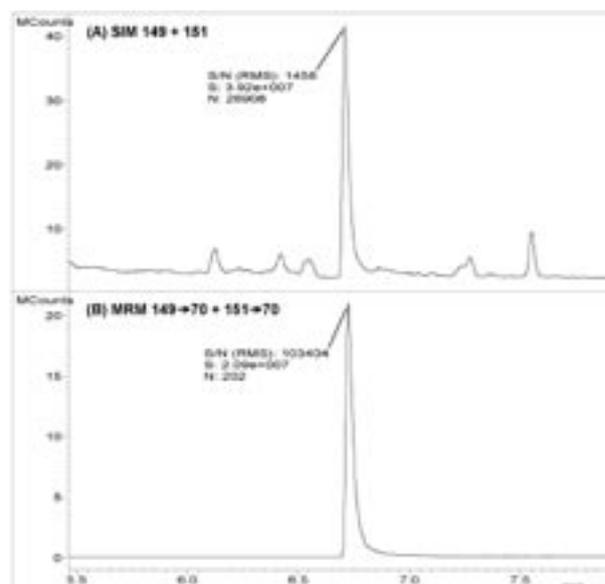


Figure 3. Increase in S/N of $\times 70$ using MRM for a fried wheat based snack containing 470 $\mu\text{g}/\text{kg}$ acrylamide

internal standard. Using multiple reaction monitoring, transitions corresponding to the loss of ^{79}Br / ^{81}Br from each parent ion were monitored. Under these conditions, a typical increase in signal/noise (S/N) of between 70 and 100 for samples and standards was observed when compared to selected ion monitoring (Figure 3). Calibration standards were linear over the range 1–1000 ng/ml ($R^2 = 0.999$). The estimated limit of detection for standard solutions of acrylamide was 0.01 ng/ml (S/N = 3).

Method performance. Although no statistical difference (ANOVA) was obtained from samples

Table 1. Recovery of acrylamide from cereal products

Sample	<i>n</i>	Acrylamide added ($\mu\text{g}/\text{kg}$)	Mean acrylamide recovered ($\mu\text{g}/\text{kg}$)	Mean recovery (%)	SD ($\mu\text{g}/\text{kg}$)	CV (%)
Bread crumb	3	0	18	–	1.0	5.6
Bread crumb	5	25	45	106	1.1	4.1
Bread crumb	2	50	66	97	2.6	5.4
Bread crumb	2	100	117	99	3.7	3.8
Bread crumb	6	1000	1054	104	11.8	1.1
Bread + acrylamide (added pre-bake)	4	–	919	–	11.4	1.2
FAPAS crispbreada	8	–	715	–	23.4	3.3

^aassigned value was 707 $\mu\text{g}/\text{kg}$

prepared with and without Carrez clarification, the treatment was retained to minimise co-extractive materials [10] and maintain robust chromatographic performance. Accuracy and precision were assessed using spiked breadcrumb, bread with added acrylamide (pre-bake) and FAPAS (crispbread) test material (Table 1). Mean recoveries from breadcrumb spiked in the concentration range (0–1000 µg/kg) were 97–106% and the mean value obtained for the FAPAS test material (715 µg/kg, CV 3.3%) was in good agreement with the assigned value (707 µg/kg).

CONCLUSIONS

A quantitative bromination of acrylamide in aqueous cereal extracts can be obtained within 25 min at room temperature. Increased sensitivity and selectivity can be achieved by using GC/MS/MS and MRM without the requirement for tedious sample preparation procedures.

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