ENVIRONMENTAL ANALYSIS

DIRECT GC DETERMINATION OF ACRYLAMIDE IN WATER USING THE AGILENT 7000B TRIPLE QUADRUPOLE GC/MS

Solutions for Your Analytical Business

Markets and Applications Programs

Authors

Jennifer P. Pascali & Michele Pozzebon

dtoLABS, Analytical Excellence Centre, Authorized Agilent Technologies Partner Lab, Resana (TV), Italy.

Francesco Spadola, CSD Product Specialist, France

Gary Lee, GC Columns Product Marketing Manager, Agilent Technologies, USA.

Abstract

Acrylamide (C3H5NO) is a polar, low MW molecule and its analysis by direct GC/MS is not an easy task, mainly because of low peak efficiency in chromatographic separation and no significant, low mass fragmentation in MS detection. With regard to these difficulties, direct GC/MS acrylamide analysis has never become a routine method and therefore, acrylamide is normally analyzed by GC/MS after derivatization (brominated) to limit strong peak broadening and increase the identification power of ion fragments.

The aim of this study was to demonstrate the possibility of carrying out reliable direct GC analysis of acrylamide, by employing Ultra Inert technology and different chemically bonded GC columns. As reliable and sensitive MS/MS detection was used, there was no need for derivatization. Standard solutions were prepared both in ethyl acetate and methanol so as to be easily coupled to SPE sample preparation. Studies were conducted on acrylamide standard solutions in the concentration range of 1-1000 ng/mL. A GC/MS method was developed and four different chemically bonded GC columns were tested in terms of peak shape, linearity, S/N, absolute areas, absolute RTs and carry over.

INTRODUCTION

The discovery in 2002 of the presence of acrylamide in various heat processed starch-rich foodstuffs by the Swedish National Food Administration, as a consequence of the Maillard reaction [1], resulted in an urgent requirement for the development of reliable analytical procedures to determine this potentially carcinogenic contaminant in a wide range of various matrices [2]. As a consequence of serious health concerns related to the dietary intake of this hazardous chemical, implementation of relevant analytical procedures became an urgent task.

Acrylamide (C3H5NO) is a polar, low MW molecule and its analysis by direct GC/MS is not an easy task, mainly because of low peak efficiency in chromatographic separation and no-significant low mass fragmentation in MS detection [3]. With regard to these difficulties, direct GC/MS acrylamide analysis has never become a routine method and therefore, acrylamide is normally analyzed by GC/MS after derivatization (brominated) to limit strong peak broadening and increase the identification power of ion fragments.

The aim of this study was to demonstrate the possibility of carrying out reliable direct GC analysis of acrylamide, by employing Ultra Inert (UI) technology and different chemically bonded GC columns. As reliable and sensitive MS/MS detection was used, there was no need for derivatization. Standard solutions were prepared both in ethyl acetate and methanol so as to be easily coupled to SPE sample preparation. Experiments were conducted on acrylamide standard solutions in the concentration range of 1-1000 ng/mL.

Four different GC columns were tested, two DB polysiloxane phases (low polarity 5% phenyl and cyanopropyl-phenyl) and two WAX phases (standard bonded polyethylene glycol and acid modified FFAP). The UI technology proved to be effective in limiting acrylamide absorption at the inlet level, and for this reason, all liners that were used were UI. A GC/MS method was developed and the four GC columns were evaluated in terms of peak shape, linearity, S/N values, reproducibility of absolute areas and RTs, and carry over effects.

EXPERIMENTAL

Materials

Acrylamide and acrylamide-d3 were purchased from Ultra Scientific (North Kingstown, RI, USA). Methanol and ethyl acetate were of MS-analytical grade from Sigma Aldrich (St. Louis, MO, USA).

Instrumentation	
Gas Chromatograph	Agilent 7890A
Autosampler	Agilent 7693
Mass Selective Detector	Agilent 7000B Triple Quadrupole
Columns	DB-5MS UI 30 m x 0.25 mm x 0.50 µm Part Number 122-5536UI
	VF-WAXms 30 m x 0.25 mm x 0.25µm Part Number CP9205
	CP-Wax 58 FFAP CB 25 m x 0.20 mm x 0.30 µm Part Number CP7787
	DB-624 UI 30 m x 0.25 mm x 1.40 µm Part Number 122-1334UI
Liners	Ultra Inert Splitless Liner Part Number 5190-3162 (5Pk) or 5190-2292 (1Pk)
	Non-deactivated Liner Part Number 19251-60540
Syringe	10µl Part Number 5190-1483
Vials	Amber Screw Top Part Number 5182-0716

Chromatographic Conditions		
Column	DB-5MS UI	WAX & DB-624 UI
Initial isotherm	50°C for 2 min	70°C for 1 min
Rate 1	45°C/min to 250°C	20°C/min to 240°C
Rate 2	30°C/min to 260°C	-
Final isotherm	260 °C for 1.22 min	240 °C for 10.5 min
Run Time	8.0 min	20.0 min
Flow	1 mL/min	1 mL/min
Injector Mode	Pulsed Splitless, 30 psi / 0.5 min, 1 µL	Pulsed Splitless, 30 psi / 0.5 min, 2 µL
Injector Temp	250°C	230°C
Transfer line Temp	260°C	240°C

MS Conditions		
Source Temperature	250°C	
Quad Temperature	150°C	
Acquisition Mode	EI MRM	
MRM Transitions	Acrylamide	71 → 55 @ 7 V
	Acrylamide	71 → 44 @ 25 V
	Acrylamide–d3	74 → 58 @ 7 V
	Acrylamide–d3	74 → 30 @ 20 V
Solvent Delay	3.6 min (DB-5MS UI) or 5 min (WAX & DB-624 UI)	

Standards

Using a 1 mg/mL stock acrylamide solution, standard solutions were prepared in ethyl acetate or methanol at concentrations of 1, 5, 10, 50, 100 and 1000 ng/mL. Deuterated acrylamide (acrylamide-d3) was used at a concentration of 10 ng/mL as a reference peak, but not for quantitation.

Solvents

Methanol and ethyl acetate were used to prepare standard solutions. Ethyl acetate was used for needle washing during analysis.

Software

Data was acquired and analyzed using the MassHunter acquisition, qualitative and quantitative platforms.

Results and Discussion

The first part of this study was to investigate the contribution of Ultra Inert (UI) Technology to peak reproducibility and analysis stability. In this regard, non-deactivated and ultra inert splitless liners were used for the analysis of a 10 ng/mL acrylamide standard solution on the developed GC method. Peak asymmetry could be attributed to the used GC-phase, but the progressive peak disappearance experienced in Fig. 1 was completely avoided by using UI liners (Fig. 2). For this reason, UI splitless liners, without wool, were used throughout this study (Fig. 3).

The second part of this study was to define the best GC column for the direct analysis of acrylamide. A total of four columns were tested:

1. Agilent DB-5MS UI (30 m x 0.25 mm x 0.50 µm)
2. Agilent VF-WAXms (30 m x 0.25 mm x 0.25 µm)
3. Agilent CP-Wax 58 FFAP CB (25 m x 0.20 mm x 0.30 µm)
4. Agilent DB-624 UI (30 m x 0.25 mm x 1.40 µm)

Each column was evaluated in terms of:

- Peak shape and absolute response (tailing factor and symmetry)
- Linearity (1-1000 ng/mL)
- Reproducibility of absolute areas and RTs
- S/N at a fixed concentration of acrylamide (5 ng/mL)
- Carry over in a blank injection after the highest concentration standard

In order to evaluate absolute responses, internal standard correction was not applied. According to the GC column specifications, the chromatographic method, especially temperatures and rates, were adapted to each column.

Peak shape and absolute response (tailing factor and symmetry)

Peak shape and absolute response were evaluated in the 10 ng/mL acrylamide standard solution (Fig. 4). The symmetry of the peak, computed as the ratio between the front half-width and the back half-width was calculated according to the USP. A symmetrical peak will have a value of 1.0. The tailing factor, defined as the ratio between the 5% widths at the base of the peak (Fig. 5), was calculated by the MH qualitative platform using the MS/MS integrator. Results for symmetry and tailing factor for each column are reported in Table 1.

The best results, in terms of symmetry and hence tailing factor, were obtained with the CP-WAX 58 FFAP CB column. Good results were obtained with the VF-WAXms and DB-624 UI columns, while the peakshape performance was much less acceptable with the low polarity DB-5MS UI column, as clearly visible in Fig. 4. Considering the molecular structure and polarity of acrylamide, this work confirms that the use of the more polar stationary phase is appropriate.

Linearity and carry over effect

Method linearity was tested with the standard solutions in the 1-1000 ng/mL concentration range using the optimized GC/MS method for each column. Two repetitions were run for each level. Calculations, made automatically by the MassHunter quantitative platform, were considered effective when achieved accuracies were in the range of 80-120% and the quantifier/qualifier area ratios were consistent with the acceptance criteria. Linear equations and R2 values, as evaluation parameters of fitness, are summarized in Table 2. In Fig. 6 an example of a linear calibration curve for the DB-624 UI column is reported. No carry-over effects were observed for any of the tested columns, after the injection of the highest tested concentration standard.

Reproducibility and S/N values

Method reproducibility, in terms of absolute areas, RTs and S/N for the quantifier MRM acrylamide transition, was calculated at the 5 ng/mL level, with 8 repetitions over two consecutive days. Absolute areas were in the range of 11853 (DB-624 UI) to 22835 (DB-5MS UI), and RTs were in the range of 4.5 (DB-5MS UI) to 8.1 (VF-WAXms). The data was collectively evaluated in terms of %RSD for each method / column set up and is summarized in Table 3. The best results for all the tested parameters were achieved with the WAX columns, with %RSD for RT below 0.04% and for absolute areas below 1.4%. The DB polysiloxane columns exhibited lower reproducibility for RTs (> 0.07%) and areas (> 1.4%), and furthermore displayed lower S/N values in this study. Noise was calculated in a 3 minute long region of clean chromatogram, using the Root Mean Square (RMS) algorithm for the 71 → 55 m/z MRM transition (Fig. 7). Calculations were automatically performed by the MassHunter software.

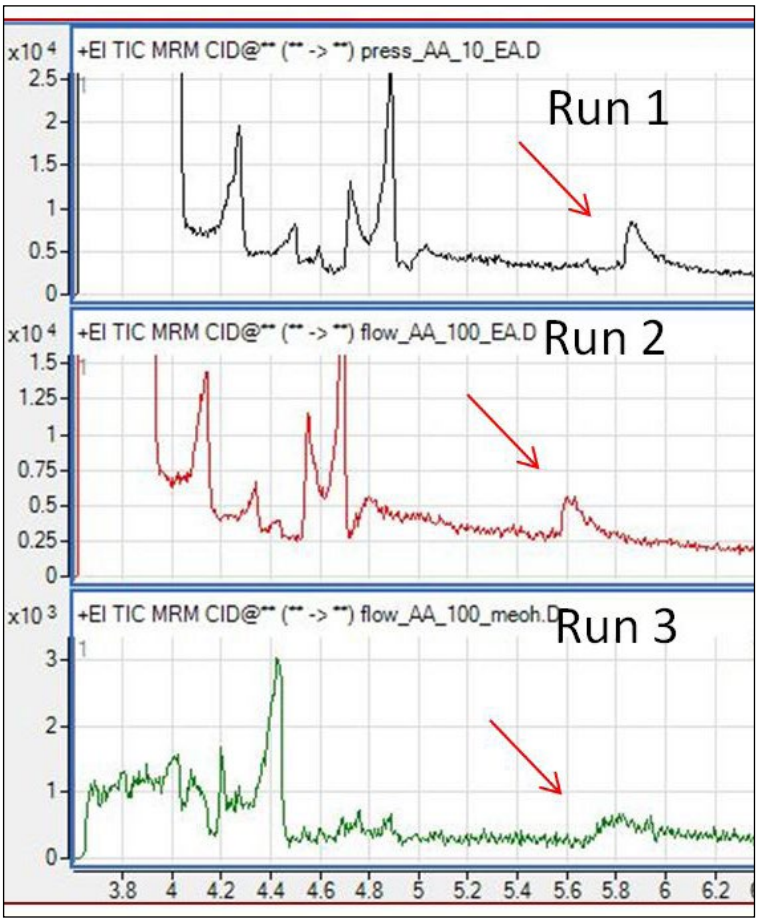


Figure 1. Consecutive injections of acrylamide at the concentration of 10 ng/mL in ethyl acetate. The use of non-deactivated inlet liners produced extensive peak broadening and subsequent runs were not reproducible.

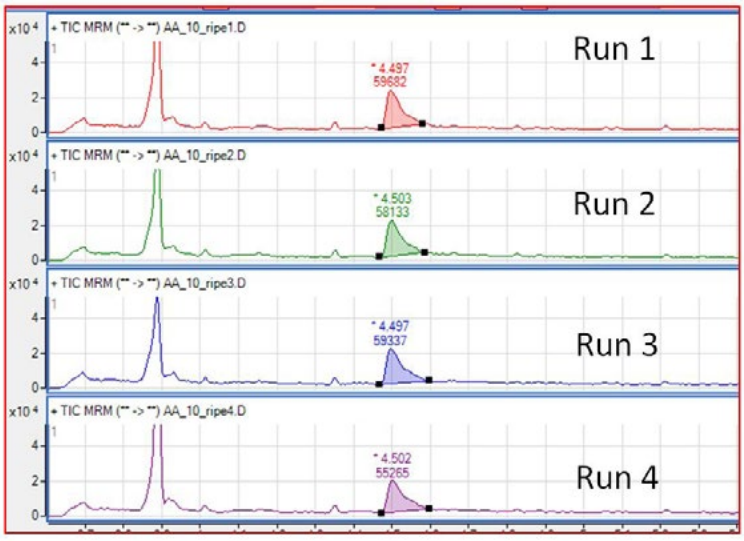
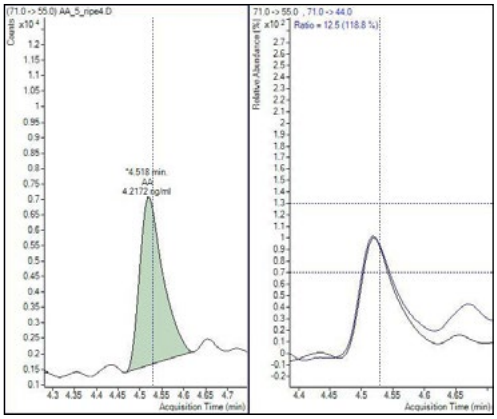


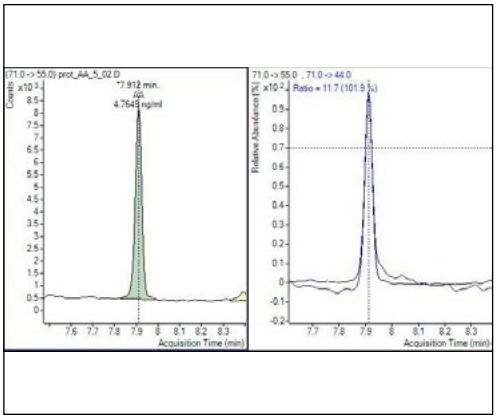
Figure 2. Consecutive injections of acrylamide at the concentration of 10 ng/mL in ethyl acetate. The use of Ultra Inert deactivated inlet liners reduced peak disappearance and improved overall instrument reproducibility in subsequent runs.

Figure 3. Ultra Inert Inlet Liners - Splitless, Single Taper, No Wool.

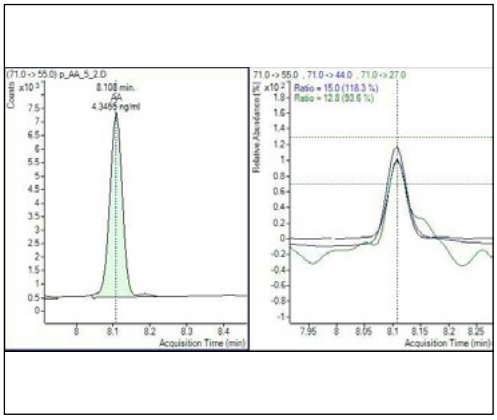
1. DB-5MS UI



3. CP-Wax 58 FFAP CB



2. VF-WAXms



4. DB-624 UI

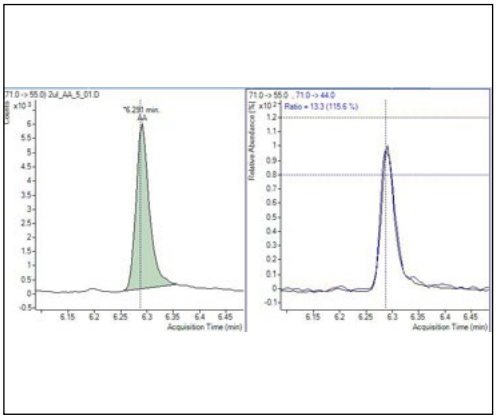


Figure 4. Acrylamide peak shape for each column.

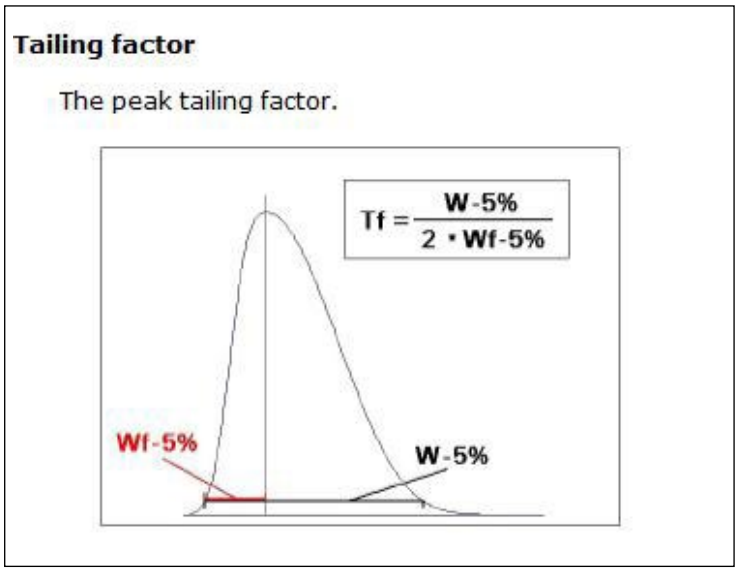


Figure 5. Calculation of the peak tailing factor.

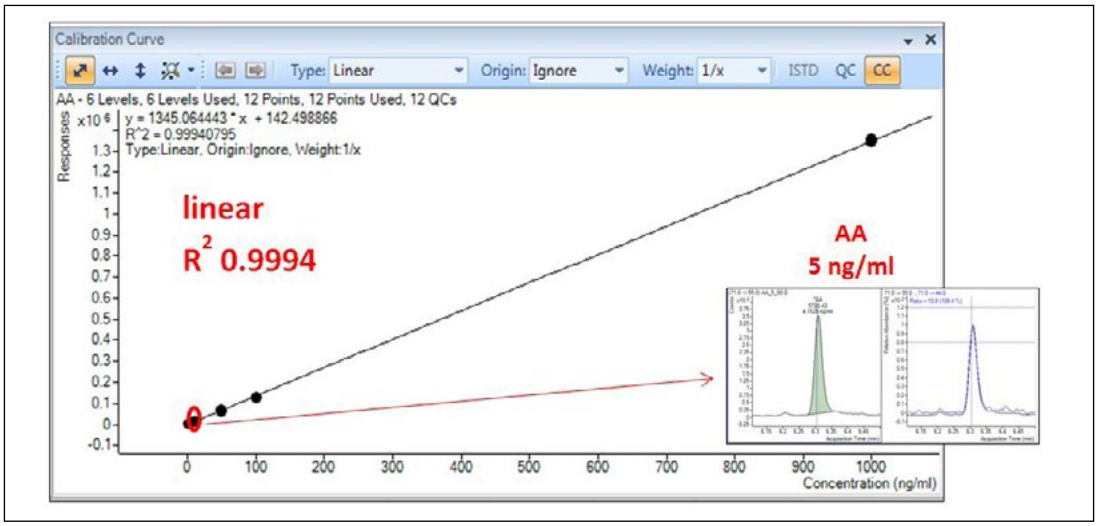


Figure 6. Calibration curve obtained for acrylamide with the DB-624 UI column in the range of 1-1000 ng/mL (6 levels, 2 repetitions per level) and the chromatogram of the acrylamide peak at 5 ng/mL.

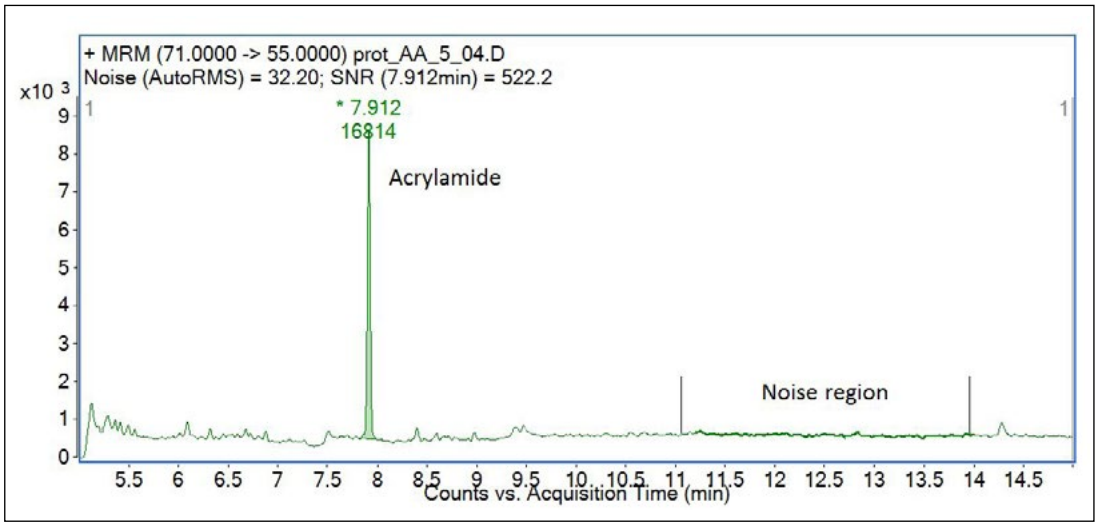


Figure 7. S/N calculation for the acrylamide peak. The noise region is highlighted.

Column type	Tailing factor	Symmetry
DB-5MS UI	6.4	0.09
VF-WAXms	1.1	0.6
CP-Wax 58 FFAP CB	0.9	1.33
DB-624 UI	1.7	0.5

Table 1. Summary of results for peak tailing and symmetry for each column.

	DB-5MS UI	VF-WAXms	CP-Wax 58 FFAP CB	DB-624 UI
Equation	Y=6829X -5559	Y= 2362X+ 6128	Y= 3539X -73	Y= 1345X+142
R2	0.998115	0.999967	0.999929	0.9994

Table 2. Summary of equations and R2 values for each column.

	Mean RT (% RSD)	% RSD Absolute Area	Mean S/N (% RSD)
DB-5MS UI	4.52 (0.07)	7	13 (22)
VF-WAXms	8.1 (0.04)	1.4	214 (29)
CP-Wax 58 FFAP CB	7.91 (0.0002)	0.5	570 (19)
DB-624 UI	4.77 (4)	4	34 (14)

Table 3. Summary of RT, absolute area and S/N data for the 5ng/mL acrylamide standard.

Conclusion

As concluding remarks, it can be stated that better analytical performance was achieved with the high polarity WAX type columns rather than the low polarity polysiloxane-type columns for the tested parameters when analyzing acrylamide.

Analytical performance of the VF-WAXms and CP-Wax 58 FFAP CB columns was substantially equivalent. However, the CP-Wax 58 FFAP CB column could be considered the column of choice when analyzing acrylamide by direct GC-MS/MS, since it showed good absolute RT stability, acceptable peak symmetry and low %RSD absolute area in repeated injections. Ensuring an inert GC flow path, with respect to the injection port liner, is a key aspect in obtaining optimum system performance.

References

1. Richard H. Stadler, Imre Blank, Natalia Varga, Fabien Robert, Jörg Hau, Philippe A. Guy, Marie-Claude Robert, Sonja Riediker, "Acrylamide from Maillard reaction products", Nature, 419, 449, 2002.

2. David Sharp, "Acrylamide in food", The Lancet, 361, 361, 2003.

3. Lenka Dunovská, Tomáš Čajka, Jana Hajšlová, Kateřina Holadová, "Direct determination of acrylamide in food by gas chromatography–high-resolution time-of-flight mass spectrometry", Analytica Chimica Acta, 578, 234, 2006.

Solutions for your analytical business
Markets & Applications Programs
www.solutions-to-win.com

The Measure of Confidence

Agilent Technologies

Published in USA, October, 2014
5991-5297EN