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Determination of free melamine content in uncured non-ionic and sulfonated melamine-based resins by reversed-phase and mixed-mode liquid chromatography

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ABSTRACT

Melamine-formaldehyde resins are used in various industrial applications. As melamine is a toxic compound on the candidate list of Substances of Very High Concern, its content in melamine-based resins is important. Here we present two high-performance liquid chromatography methods suitable for the determination of free melamine content in uncured non-ionic and negatively charged melamine-based resins. A gradient reversed-phase liquid chromatography method using a C18 column was developed for the determination of free melamine content in non-ionic resins, while an isocratic mixed-mode liquid chromatography method using a Primesep 100 column was developed for the quantification of free melamine in sulfonated melamine-based resins. Both methods utilise ultraviolet detection, which allows the quantification of melamine in melamine-based resins with a limit of quantification of $\sim 10^{-3}$% by weight and good repeatability and linearity over a wide range of melamine concentrations.

1. Introduction

Polycondensates of melamine and formaldehyde (MF resins) are used in various applications, e.g. in coating systems for the automotive industry, in the construction industry to improve the quality of concrete and in the food industry to improve the heat-resistance of tableware, dishes, cups, etc. [1,2]. Since melamine (2,4,6-triamino-1,3,5-triazine) is toxic [3], it is important to know the content of free melamine in melamine-based resins.

The studies on MF resins mainly focused on determining the amount of migrated melamine from cured MF resins under different conditions, i.e. temperature, media type, pH, ultraviolet (UV) radiation, etc. Under certain conditions, melamine was extracted from kitchen utensils or food packaging and quantified by gas chromatography with selective nitrogen-phosphorous detector [2] or by high-performance liquid chromatography (HPLC) with UV or mass spectrometer (MS) as detector [3–16]. HPLC studies on the quantification of migrated melamine have been performed on reversed stationary phase columns (C18 [4–8] and C8 [9]) using acidic pH mobile phases, HILIC columns [7,10–14] and mixed-mode stationary phase columns [3,7,15,16]. While most studies dealing with the determination of free melamine have been performed on cured MF resins, investigations on uncured, soluble resins are still limited [17–21].

The structure of MF resins and the amount of free melamine in the range of 6 – 20% by weight (wt%) in aqueous solutions of uncured, non-ionic melamine-urea-formaldehyde resins were studied by ^{13}C NMR spectroscopy [17]. The type of monomeric, dimeric and oligomeric species in water-soluble non-ionic MF resins was identified by capillary zone electrophoresis coupled to an MS detector [18] and HPLC on a C18 reversed stationary phase with UV and MS detectors [19], but both studies provided only qualitative information on the type of the species composing the MF resins without their quantification. The relative amounts of simple methylol melamines including melamine in uncured non-ionic MF resin were determined by gradient reversed-phase liquid chromatography (RPLC) with a UV detector and methanol / water mobile phase, although melamine was not baseline separated from the other species [20]. The amount of free melamine in water-soluble MF resins was also evaluated by Raman spectroscopy and gradient RPLC-UV on a C18 reversed stationary phase with acetonitrile (ACN) / water mobile phase [21]. The results of both techniques agree very well for melamine contents in the range of 9 – 40 wt% [21].

Most studies on MF resins have mainly focused on determining the amount of migrated melamine from the cross-linked MF resins under different conditions, while studies on the free melamine content in the

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uncured non-ionic MF resins are rare and limited to a few wt%, while no studies are yet available for negatively charged MF resins. As melamine has recently been added to the list of substances of concern [3,10, 13–16], it is to be expected that companies producing melamine-based resins will sooner or later have to declare the free melamine content in their products. Our aim was therefore to develop fast and simple methods with a low limit of quantification (down to 0.1 wt%), good repeatability and linearity over a wide range of melamine concentrations to determine free melamine content in uncured non-ionic and negatively charged MF resins of different chemical composition.

2. Materials

The samples of MF resins were supplied by Melamin d.d. (Slovenia) in the form of aqueous solutions, with the exception of MF-5 resin, which was supplied as a solution in *n*-butanol, and MF-7 resin, which was supplied in a solid state. MF-1, supplied as aqueous solution (solid content = 73 ± 2%), is a methylated resin used in paper and cardboard production to improve the physical properties of the product such as abrasion, chemical, heat and water resistance. MF-2, supplied as aqueous solution (solid content = 60 ± 2%), is an unmodified MF resin used for the impregnation of decorative papers. MF-3, supplied as aqueous solution (solid content = 76 ± 2%), is a partially etherified MF resin used in the textile industry as a crosslinking agent for polymer dispersions. MF-4 (solid content = 70 ± 2%) is a methylated MF resin used in paper and cardboard production to improve physical properties of products such as abrasion, chemical, heat and water resistance. MF-4 and MF-1 resins differ in the molar ratio between melamine and formaldehyde used in the formulation, the degree of methylation, the solid content and the molecular weight characteristics. MF-5, supplied as a solution in *n*-butanol (solid content = 71 ± 2%), is a water-insoluble *n*-butylated MF resin used in coating agents for metals and enamelware. MF-6, supplied as aqueous solution (solid content = 39 ± 2%), and MF-7, supplied in a solid state (solid content = 100%), are sulfonated MF resins used in the construction industry to improve the physical properties of concrete. ACN (≥99.9%; Riedel de Haën, Germany), high-purity Milli-Q water with a resistivity of 18.2 MΩ·cm, and trifluoroacetic acid (TFA; ≥99.5%; Arcos Organics, France) were used as solvents for HPLC analyses.

3. Experimental

3.1. Reversed-phase liquid chromatography for non-ionic melamine-based resins

Separation of free melamine from other species of non-ionic MF resins was performed by gradient RPLC on a Zorbax Eclipse XDB-C18 column (4.6 mm × 150 mm, 80 Å, 5 µm, $t_0 = 1.50$ min; Agilent Technologies, USA) at 30°C in a thermostated oven. A binary HPLC pump (Agilent 1260, Agilent Technologies, USA) delivered the mobile phase at a nominal flow rate of 1 mL min^{-1}. The initial composition of the mobile phase was 100% water with a pH of 3.0 or 4.0, which was adjusted using a pH meter with glass electrode (Mettler Toledo, Switzerland) with addition of TFA. The ACN / water solvent gradients used are listed in Table 1. The supplied MF solutions were diluted with water to a concentration of ~0.5 mg mL^{-1}, except for the MF-4 resin, which was diluted to ~0.7 mg mL^{-1} due to its low melamine content. As the MF-5 resin precipitated after the addition of water, it was filtered through a polytetrafluoroethylene (PTFE) membrane filter with a pore size of 0.45 µm before being injected onto the HPLC column. The injection volume of the samples was 20 µL. A UV detector (Agilent 1260) operating at a wavelength of 210 nm was used for detection.

Table 1
ACN / water solvent gradients used in RPLC.

Solvent gradient-*a*			Optimal solvent gradient-*b*		
t [min]	%water	%ACN	t [min]	%water	%ACN
0	0	0	0	100	0
30	10	10	10	97	3
40	20	20	20	60	40
42	22	22	22	100	0

3.2. Mixed-mode liquid chromatography (MMLC) for sulfonated melamine-based resins

The determination of free melamine in solutions of negatively charged sulfonated MF resins was performed by isocratic MMLC on a Primesep 100 column (4.6 mm × 150 mm, 100 Å, 5 µm, $t_0 = 1.69$ min; SiELC Techonologies, USA) at 25°C in a thermostated oven. A binary HPLC pump (Agilent 1260, Agilent Technologies, USA) delivered the mobile phase with a constant composition of 50 vol% ACN and 50 vol% water with 0.10 vol% TFA at a nominal flow rate of 1 mL min^{-1}. The MF-6 solution was diluted with a 50 / 50 vol% mixture of ACN and pure water. The MF-7 supplied as a solid was dissolved in the same solvent mixture to prepare a MF-7 solution with a concentration of ~4 mg mL^{-1}. The injection volume of the samples was 20 µL. A UV detector (Agilent 1260) operating at a wavelength of 210 nm was used for detection.

3.3. Quantification of free melamine in melamine-based resins

The quantification of free melamine in MF solutions was performed using a calibration curve representing the area under the melamine peak in the UV-chromatograms of the melamine standard solutions of different concentrations as a function of the injected melamine mass at an injection volume of 20 µL. The calibration curve for the non-ionic MF resins with high free melamine content was constructed from melamine standard solutions with concentrations ranging from 2.16×10^{-3} to 43.3 µg mL^{-1} (Figure S1a) and from 2.16×10^{-3} to 0.108 µg mL^{-1} for the MF resins with low free melamine content (Figure S1b). The calibration curve for the sulfonated MF resins was constructed from melamine standard solutions with concentrations ranging from 0.021 to 0.171 µg mL^{-1} (Figure S1c). Each standard solution was injected onto the column in triplicate to obtain an average area under the peak of the melamine standard. From the peak areas corresponding to free melamine in the chromatograms of the MF resins, its content in the original aqueous or *n*-butanol MF solutions was determined from the corresponding calibration curve, while in the case of MF-7, the free melamine content is reported per neat MF since it was supplied in solid state. From the peak areas corresponding to the free melamine in the chromatograms of the MF resins, its content per resin was also determined considering the solids content of the original resin solutions given by the resin manufacturer (Eqs. S1 – S5).

3.4. Validation of chromatographic methods

3.4.1. System repeatability

The system repeatability test of the developed HPLC methods was performed by six consecutive injections of the melamine standard solution with known concentration and the determination of the area under the melamine peak recorded by the UV-detector for each injection (A_i). The relative standard deviation in percent (%RSD) of the peak area was then calculated according to Eq. (S6). The acceptance criteria for a passed system precision test is %$RSD \leq 2\%$.

3.4.2. Linearity

The linearity of the HPLC methods developed was assessed from the coefficient of determination or R-squared value (R^2) of the calibration curves (Figure S1). The acceptance criteria for R^2 is ≥0.99.

3.4.3. Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) were determined on the basis of a statistical approach for the corresponding calibration curve. LOD and LOQ were determined from the slope (*S*) and the standard deviation of the intercept of the calibration curve (α) according to the Eqs. S7 – S9.

4. Results and discussion

4.1. Non-ionic melamine-based resins

The baseline separation of melamine from other components of MF resins is a prerequisite for the accurate quantification of free melamine by HPLC. For this purpose, various experimental parameters were optimised, e.g. the steepness of solvent gradient in RPLC, the amount of acidic modifier (TFA) added to the water component of the mobile phase and sample preparation procedure.

4.1.1. pH value of the water component of the mobile phase

Melamine with a pK_a of 5 is a basic compound. Under neutral conditions, its amino groups (–NH$_2$) interact with the remaining silanol groups of the C18-modified silica-based stationary phase, resulting in non-reproducible chromatograms and mass recovery of the melamine from the column, making its quantification unreliable [22,23]. By adding an acidic modifier (TFA) to the mobile phase, the amino groups are converted to their protonated form (–NH$_3^+$), preventing their interaction with the silanol groups of the C18-modified silica-based stationary phase [24,25]. The melamine standard was dissolved in pure water and analysed by gradient RPLC using a mobile phase consisting of ACN and water with a pH of 3 or 4. At a pH of 3 for the water component of the mobile phase, the melamine retained in the column ~0.2 min longer than at a pH of 4 (2.17 min vs. 1.97 min; Figure S2), which can be attributed to the chaotropic effect of the CF$_3$COO$^-$ ions of TFA [25].

The same experiment was performed with non-ionic MF-4 resin diluted with pure water prior to HPLC analysis (Fig. 1). The enlarged chromatograms of the MF-4 resin in the region where melamine elutes show that melamine is well separated from the other resin components at a water pH of 3 (Fig. 1b). In addition, a comparison of the chromatograms in the retention time range of 12.5 – 22.5 min obtained at water pH 3 and 4 shows a higher degree of degradation of MF resin at lower pH (Fig. 1a), which can be attributed to the acid-catalysed hydrolysis of ether bridges in the resin structure [26] as well as to the fact that in gradient RPLC macromolecules are separated not only by their chemical composition but also by their molar mass [27]. Although the MF resins degrade faster under more acidic conditions, the determined free melamine content in the MF-4 solution is slightly higher at pH 4 than at pH 3 (5.98 × 10^{-3} wt%) at pH 4 vs. 4.31 × 10^{-3} wt% at pH 3), which is due to insufficient separation of the free melamine from the other resin components at pH 4 (Fig. 1b). Based on these results, we chose a pH of 3 for the water component of the mobile phase for further work.

4.1.2. Solvent gradient

To make the method for quantifying free melamine in MF resins efficient, the method must be short due to the instability of MF resins in acidic solutions. At a pH of 3 of the water component of the mobile phase and using a smooth gradient of ACN in water, the non-ionic MF resins eluted from the column in a wide retention time range (Fig. 2a), indicating a better separation of the individual resin components compared to the steeper gradient where the resin components eluted from the column faster and in a much narrower retention time range (Fig. 2b). Nevertheless, both solvent gradients baseline separated the free melamine from the other resin components (enlarged chromatograms in Fig. 2). Moreover, the elution time of melamine depends only slightly on the slope of the gradient (Figure S3). Since our aim was to sufficiently separate free melamine from other resin components, a steeper gradient was chosen for further experiments, which allows for a shorter analysis time (Fig. 2b).

4.1.3. Sample preparation procedure

MF resins degrade over time in aqueous solutions, especially under acidic conditions [26,28]. Therefore, the pH of the water used to dilute / dissolve MF resin prior to HPLC analysis plays an important role in sample preparation. The chromatograms of the two consecutive measurements of the MF-4 resin diluted with water (without added TFA) and analysed immediately after mixing on a vortex for 15 s (first run) and after ~25 min (second run) are fairly comparable (Fig. 3a). However, this is not the case when the resin was diluted with water of pH 3 (Fig. 3c). Compared to the first run, the chromatogram of the second run in Fig. 3c shows a higher intensity of the peaks labelled 1-4 and a lower intensity of the peaks between 16 – 20 min, which is due to the resin degradation. Two consecutive measurements of the same sample solution diluted with water of pH 3 after standing at room temperature for 3 h show more comparable chromatograms (Fig. 3d), indicating extensive degradation of specific bonds in the structure of the MF resin. The area of the melamine peak in the chromatograms of the MF-4 resin depends less on whether the sample was diluted with water or with water at pH 3 when the sample is measured immediately after dilution (Fig. 3a, c, Table 2). In contrast, after storing the resin for 3 h, the area under the melamine peak and thus the determined free melamine content increased independently of the pH of the water, but to a much greater

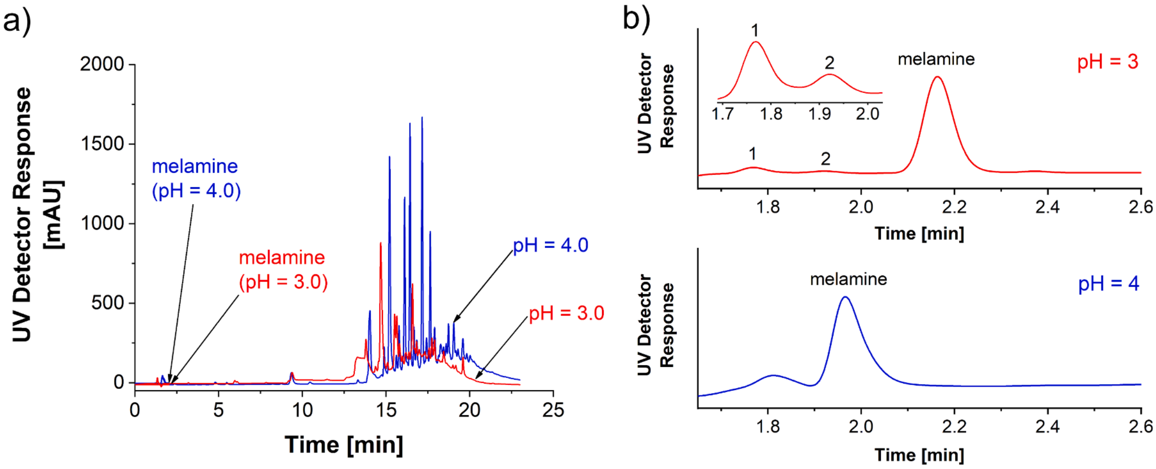

Fig. 1. a) Gradient RPLC-UV chromatograms of the non-ionic MF-4 resin on the Zorbax Eclipse XDB-C18 column at an optimised solvent gradient and a pH of the water component of the mobile phase of 3 and 4. b) Enlarged gradient RPLC-UV chromatograms of the MF-4 resin in the region where melamine elutes. The column temperature was 30°C and the flow rate of the mobile phase was 1 mL min^{-1}.

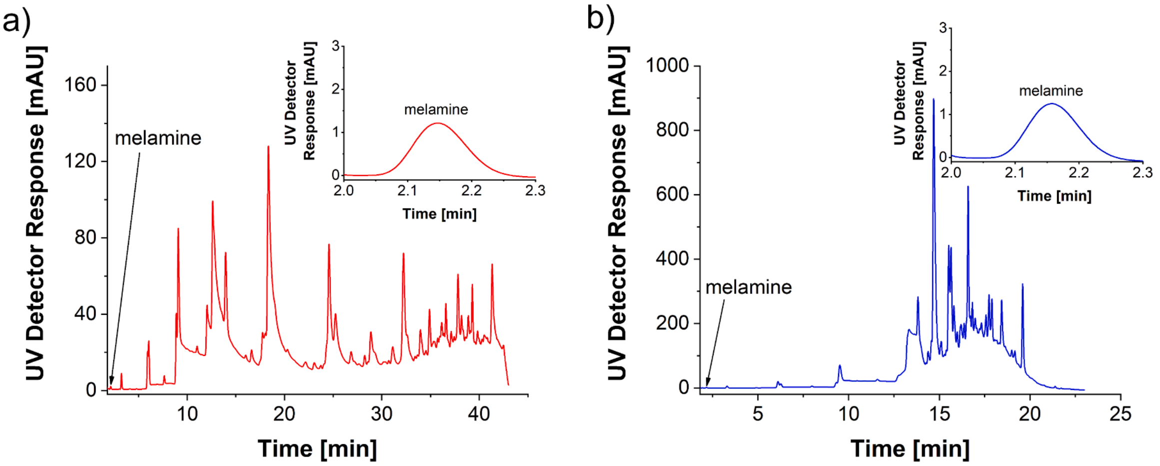

Fig. 2. Gradient RPLC-UV chromatograms of the non-ionic MF-4 resin on the Zorbax Eclipse XDB-C18 column at a pH of the water component of the mobile phase of 3 and different solvent gradients given in Table 1, i.e. a) solvent gradient-a and b) optimal solvent gradient-b. The column temperature was 30°C and the flow rate of the mobile phase was 1 mL min^{-1}.

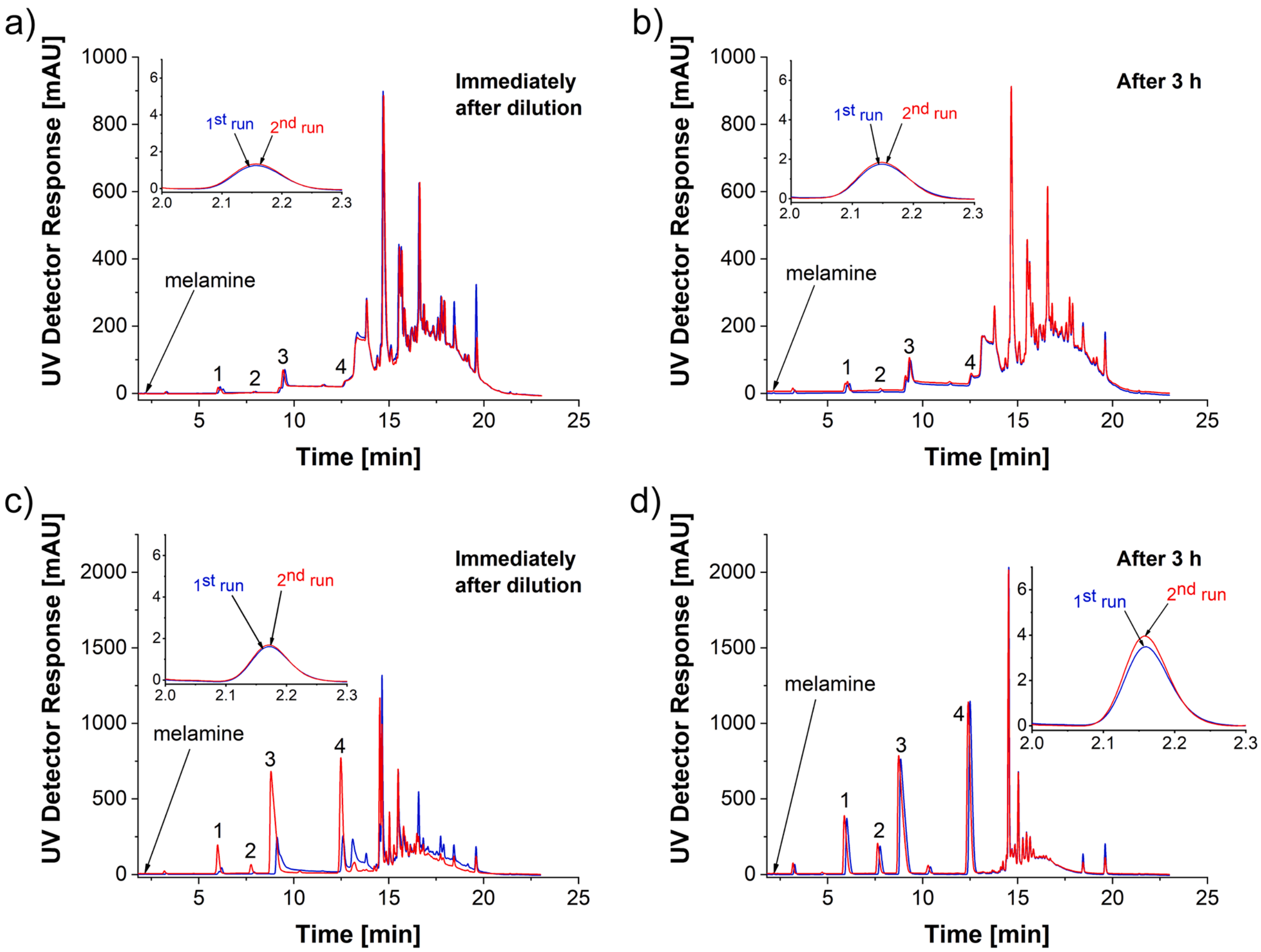

Fig. 3. Gradient RPLC-UV chromatograms of the non-ionic MF-4 resin on the Zorbax Eclipse XDB-C18 with enlarged regions where melamine elutes. The sample was injected onto the column immediately after dilution with water (a) or water at pH 3 (c) and after standing for three hours at room temperature (b, d). In all cases, two consecutive measurements were performed with an optimised solvent gradient. The column temperature was 30°C and the flow rate of the mobile phase was (1 mL min^{-1}).

extent when the MF resin was stored under acidic conditions (Fig. 3b, d, Table 2), again indicating accelerated degradation of MF resins under acidic conditions. Due to the poor stability of MF resins under acidic conditions, they were dissolved / diluted with water when dilution was required prior to HPLC analysis.

Under optimised conditions, the amount of free melamine was determined for non-ionic MF resins with different chemical compositions (Table 3). Depending on the melamine content, the MF solutions

Table 2
The free melamine content in the non-ionic MF-4 resin solution as determined by diluting the resin with water or water at pH 3 immediately after dilution and after 3 h.

Dilution time	Run	$C_{\text{MFsolut.}}$ [mg mL^{-1}]	Peak Area [× 10^{-3}]	Free Melamine Content[1] [wt% × 10^{-3}]
MF diluted with water				
15 s	1st run	0.703	0.1205	4.31
	2nd run	0.703	0.1274	4.51
3 h	1st run	0.703	0.1659	5.59
	2nd run	0.703	0.1738	5.82
MF diluted with water at pH 3				
15 s	1st run	0.703	0.1302	4.58
	2nd run	0.703	0.1302	4.58
3 h	1st run	0.703	0.2664	8.44
	2nd run	0.703	0.2998	9.38

[1] Determined based on calibration curve in Figure S1b.

were diluted appropriately with water just before the HPLC measurements. After the addition of water to the MF-5 solution in *n*-butanol, the sample partially precipitated, which is why it was filtered prior to injection onto the column. The results show good repeatability of the melamine content between two resin solutions prepared in parallel and also good repeatability between parallel runs (Table 3), confirming the suitability (Table S1, Figure S4) of the developed method for determining the free melamine content even for concentrations as low as ~10^{-3} wt%.

4.2. *Sulfonated melamine-based resins*

The developed RPLC method is unsuitable for the determination of free melamine in negatively charged sulfonated MF resins (Figure S5a) because the melamine peak is not well separated from the components of the sulfonated MF resin, which hardly interact with the non-polar C18 stationary phase due to the polar nature of the resin (Figure S5b). To improve the separation of melamine from the components of sulfonated MF resins, we used MMLC on a Primesep 100 column and ACN / water / TFA mobile phase with a constant composition. The stationary phase of the Primesep 100 column is modified by alkyl and carboxyl groups and separates the analytes according to the mechanism of hydrophobic interaction and cation-exchange, so that the retention of basic compounds depends on the volume ratio between ACN and water and the amount of acid modifier added [29–31]. At the 50 : 50 vol% mixture of ACN and water, the retention time of the melamine standard increases with decreasing TFA concentration (from 4.8 min at 0.15 vol% TFA to 7.1 min at 0.10 vol% TFA; Figure S6), which is due to the fact that at higher TFA concentration the extent of deprotonation of the carboxyl groups embedded in the column packing material is lower [32,33], resulting in a lower probability of electrostatic interaction with protonated amino groups (–NH_3^+) and ultimately a shorter retention time of the melamine.

The same chromatographic conditions were used for the sulfonated MF resins. To avoid degradation of the MF resins, they were first diluted / dissolved with a 50 : 50 mixture of ACN and water without the addition of TFA, mixed on a vortex mixer for 15 s and immediately injected onto the column twice in succession (12 min delay time between the two injections). The negatively charged components of the sulfonated MF resins are largely not retained on the mixed-mode stationary phase due to the repulsion between negatively charged resin and stationary phase, while oppositely charged free melamine interacts with the stationary phase and is sufficiently separated from the resin components at 0.10 vol % TFA in water (Fig. 4). The optimum composition of the mobile phase for the determination of free melamine in sulfonated MF resins was found to be 50% ACN and 50% water with added TFA at a concentration of 0.10% by volume.

Under optimal conditions, good repeatability of melamine content was obtained for the solutions of MF-6 resin prepared in triplicate and

Table 3
The content of free melamine in non-ionic MF resin solutions (given in % by weight) and in neat MF resins (given in mg kg^{-1}), taking into account the solids content of the resins.

	$c_{\text{MFsolut.}}$ [mg mL^{-1}]	Peak Area [× 10^{-3}]	Free Melamine Content [wt%]
MF-1 resin[1]			
1st parallel	0.500		
1st run		62.03	2.50 ± 0.01
2nd run		62.34	2.51 ± 0.01
2nd parallel	0.508		
1st run		63.43	2.52 ± 0.01
2nd run		63.82	2.53 ± 0.01
3rd parallel	0.512		
1st run		64.51	2.54 ± 0.01
2nd run		64.92	2.55 ± 0.01
Average of all parallels [wt%]			2.53 ± 0.02
MF-1 [mg kg^{-1}]	*184.7*		
MF-2 resin[1]			
1st parallel	0.509		
1st run		167.80	6.65 ± 0.03
2nd run		169.14	6.70 ± 0.03
2nd parallel	0.505		
1st run		163.52	6.53 ± 0.03
2nd run		164.82	6.58 ± 0.03
3rd parallel	0.515		
1st run		172.52	6.75 ± 0.04
2nd run		174.11	6.81 ± 0.04
Average of all parallels [wt%]			6.67 ± 0.10
MF-2 [mg kg^{-1}]	*400.20*		
MF-3 resin[2]			
1st parallel	0.501		
1st run		0.1385	(6.8 ± 0.1) × 10^{-3}
2nd run		0.1442	(7.0 ± 0.1) × 10^{-3}
2nd parallel	0.508		
1st run		0.1634	(7.6 ± 0.2) × 10^{-3}
2nd run		0.1686	(7.9 ± 0.2) × 10^{-3}
3rd parallel	0.501		
1st run		0.1510	(7.3 ± 0.1) × 10^{-3}
2nd run		0.1557	(7.5 ± 0.1) × 10^{-3}
Average of all parallels [wt%]			(6.7 ± 0.4) × 10^{-3}
MF-3 [mg kg^{-1}]	*0.51*		
MF-4 resin[2]			
1st parallel	0.703		
1st run		0.1205	(4.3 ± 0.1) × 10^{-3}
2nd run		0.1274	(4.5 ± 0.1) × 10^{-3}
2nd parallel	0.700		
1st run		0.1262	(4.5 ± 0.1) × 10^{-3}
2nd run		0.1289	(4.6 ± 0.1) × 10^{-3}
3rd parallel	0.702		
1st run		0.1238	(4.4 ± 0.01) × 10^{-3}
2nd run		0.1244	(4.4 ± 0.01) × 10^{-3}
Average of all parallels [wt%]			(4.5 ± 0.1) × 10^{-3}
MF-4 [mg kg^{-1}]	*0.31*		
MF-5 resin[2,3]			
1st parallel	0.506		
1st run		0.0694	(4.0 ± 0.1) × 10^{-3}
2nd run		0.0733	(4.1 ± 0.1) × 10^{-3}
2nd parallel	0.511		
1st run		0.0719	(4.0 ± 0.1) × 10^{-3}
2nd run		0.0755	(4.2 ± 0.1) × 10^{-3}
3rd parallel	0.508		
1st run		0.0732	(4.1 ± 0.1) × 10^{-3}
2nd run		0.0749	(4.2 ± 0.1) × 10^{-3}
Average of all parallels [wt%]			(4.1 ± 0.1) × 10^{-3}
MF-5 [mg kg^{-1}]	*0.29*		

[1] Free melamine content was determined based on the calibration curve in Figure S1a.

[2] Free melamine content was determined based on the calibration curve in Figure S1b.

[3] Sample suspension was filtered through a PTFE membrane filter with a pore size of 0.45 μm before injection onto the C-18 column.

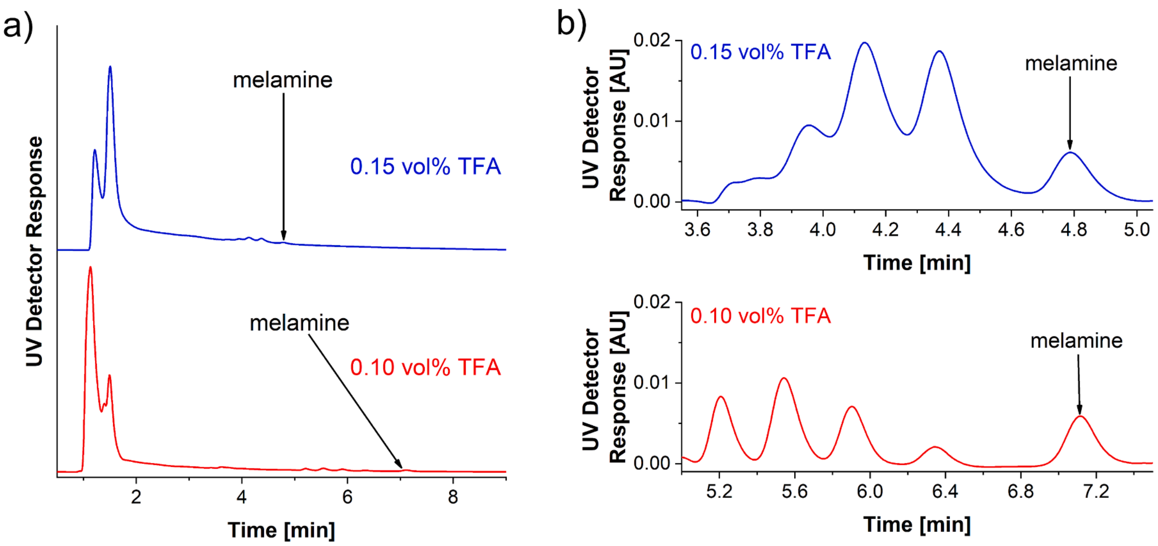

Fig. 4. a) MMLC-UV chromatograms of the negatively charged sulfonated MF-6 resin on the Primesep 100 column with b) enlarged regions where melamine elutes. Mobile phase composition: 50% ACN and 50% water with added TFA at concentration of 0.15% (top) and 0.10% (bottom) by volume. The column temperature was 25°C and the flow rate of the mobile phase was 1 mL min^{-1}.

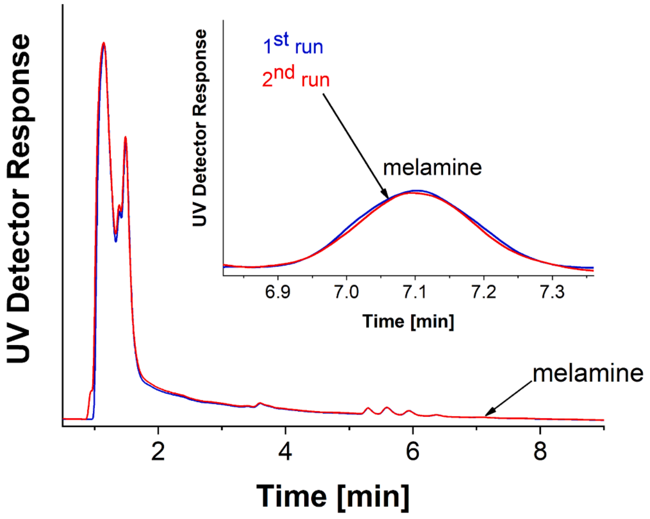

Fig. 5. Two sequential MMLC-UV chromatograms of the negatively charged sulfonated MF-6 resin with enlarged MMLC-UV chromatograms in the area where melamine elutes on the Primesep 100 column and using the mobile phase composition of 50% ACN and 50% water with added TFA at a concentration of 0.10% by volume. The column temperature was 25°C and the flow rate of the mobile phase was 1 mL min^{-1}.

also for two consecutive injections of each resin solution (Fig. 5, Table 4) as MF-6 degrades slowly. In contrast, for the MF-7 resin supplied in the solid state and further dissolved in a 50 : 50 vol% mixture of ACN and water, the repeatability of the melamine content between the resin solutions prepared in triplicate and measured immediately after dissolution (first injections) was good, but the repeatability between successive injections of the same solution was poor (Table 4), which is due to the much faster degradation of this particular resin in solution. Therefore, the results of the first injections are considered to be more representative of the initial melamine content in MF-7, while a comparison of the melamine content between the first and second injections of the same resin solution can be used to infer the rate of resin degradation in the solution. The limit of quantification for free melamine in non-ionic and sulfonated MF resins is ~10^{-3} wt% (Table S1).

Table 4
The content of free melamine in sulfonated MF-6 resin solution (given in % by weight) and in neat MF resins (given in mg kg^{-1}), taking into account the solids content of MF-6.

	$C_{\text{MFsolut.}}$ [mg mL^{-1}]	Peak Area [× 10^{-3}]	Free Melamine Content[1] [wt%]
MF-6 resin			
1st parallel	4.096		
1st run		0.4034	(2.10 ± 0.05) × 10^{-3}
2nd run		0.3846	(2.00 ± 0.05) × 10^{-3}
2nd parallel	4.103		
1st run		0.4029	(2.10 ± 0.01) × 10^{-3}
2nd run		0.4047	(2.11 ± 0.01) × 10^{-3}
3rd parallel	4.045		
1st run		0.3690	(1.95 ± 0.02) × 10^{-3}
2nd run		0.3754	(1.98 ± 0.02) × 10^{-3}
Average of all parallels [wt%]			(2.04 ± 0.06) × 10^{-3}
MF-6 [mg kg^{-1}]	*0.08*		
MF-7 resin[2]			
1st parallel	3.988		
1st run		1.3570	(7.3 ± 0.8) × 10^{-3}
2nd run		1.6470	(8.8 ± 0.8) × 10^{-3}
2nd parallel	4.001		
1st run		1.3100	(7.0 ± 0.5) × 10^{-3}
2nd run		1.4860	(7.9 ± 0.5) × 10^{-3}
3rd parallel	4.042		
1st run		1.2810	(6.8 ± 0.4) × 10^{-3}
2nd run		1.4320	(7.6 ± 0.4) × 10^{-3}
Average of 1st runs[3] [wt%]			(7.0 ± 0.2) × 10^{-3}
MF-7 [mg kg^{-}1]	*0.70*		

[1] Determined based on calibration curve in Figure S1c.

[2] MF-7 supplied in solid state was prepared by dissolving it in a 50: 50 mixture of ACN and water without adding TFA, then mixing it in a vortex mixer for 15 seconds and immediately injecting it onto the column twice in succession.

[3] Free melamine content in MF-7 is given as the average value of the first injections of three prepared resin solutions, as it degrades rapidly in solution.

5. Conclusions

The free melamine was successfully separated from other resin components by gradient RPLC on a reversed C18 stationary phase for non-ionic MF resins and by isocratic MMLC on a mixed-mode stationary phase for negatively charged sulfonated MF resins. By preventing the degradation of MF resins during sample preparation procedure allows the quantification of free melamine in melamine-based resins down to ~10^{-3} wt% using the corresponding calibration curves.

CRediT authorship contribution statement

Blaž Zdovc: Formal analysis, Investigation, Data curation, Validation, Software, Writing – original draft, Visualization, Writing – review & editing. **Ema Žagar:** Funding acquisition, Conceptualization, Methodology, Project administration, Resources, Investigation, Supervision, Data curation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of generative AI in scientific writing

The authors declare no use of any generative artificial intelligence (AI) and AI-assisted technologies in the writing process.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2025.466280.

Data availability

Data will be made available on request.

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