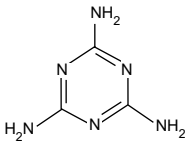
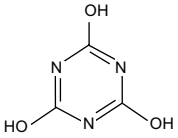
Grace Davison Discovery Sciences

HPLC and UHPLC Methods for Melamine



Melamine



Cyanuric Acid

Milk, infant formula and other dairy products were recently found contaminated with melamine, following an earlier melamine contamination outbreak in pet food in 2007.

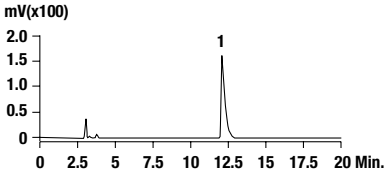
Toxicology studies show ingestion of melamine in large quantities may lead to reproductive damage or bladder cancer due to the formation of bladder/kidney stones. At lower levels, melamine and cyanuric acid are absorbed into the bloodstream. Together, they concentrate and form melamine cyanurate in the urine-filled renal microtubules. Cystallization blocks and damages the renal cells that line the tubes, causing the kidneys to malfunction.

After 2007 and more recent melamine contamination outbreaks, there is an urgent need for analytical methods that can identify and quantify melamine in food. Current melamine detection methods involve LC-MS and GC-MS. GC-MS methods require derivatization, and LC-MS methods generally use gradient conditions that require column clean up and re-equilibration.

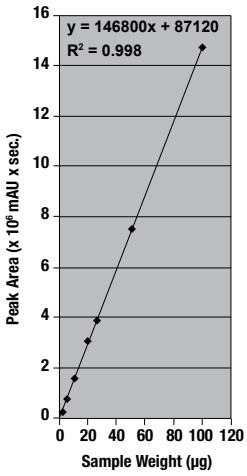
Grace has developed MS-compatible LC methods for Melamine using an HILIC media platform that can be applied to both traditional HPLC as well as UHPLC systems. Melamine was analyzed with a standard HPLC system using a 5µm particle HILIC phase packed into a 250 x 4.6mm column. The 1.5µm version of this phase was then packed into a high throughput format conducive to UHPLC and fast LC systems. Both methods deliver excellent linearity and use isocratic elution for fast analysis without the need for re-equilibration.

HPLC Method for Melamine

This HPLC analytical method for melamine fulfills the FDA requirements using a HILIC column and an ionizable mobile phase compatible with mass spec. Low UV detections offers excellent linearity between 40ng and 100µg.

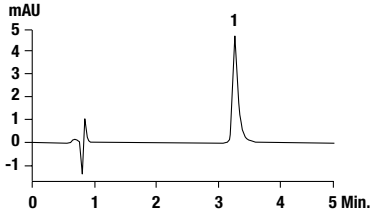


HPLC Column: VisionHT™ HILIC, 5µm, 4.6 x 250mm (Part No. **86466**)
Mobile Phase: Acetonitrile:10mM Ammonium Acetate in Water (95:5)
Flow Rate: 1mL/min
Detection: UV@240nm
Column Temperature: 30ºC
Injection: 40µg/mL x 20µL



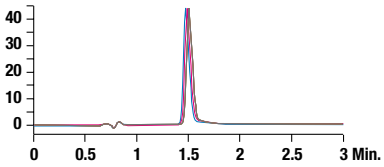
UHPLC Method for Melamine

Compared to the conventional HPLC method, the UHPLC method is 4 times faster. With the use of 1.5µm particles, optimal linear velocities extend over a wider range. Therefore, it is possible to maintain efficiency and resolution while running samples at faster flow rates.

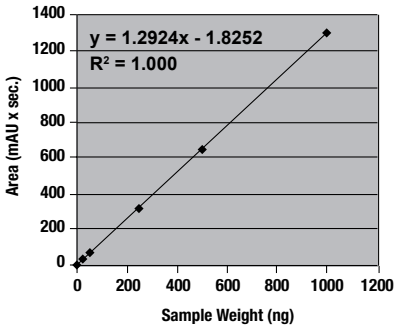


UHPLC Column: VisionHT™ HILIC, 1.5µm, 2 x 50mm (Part No. **5141919**)
Mobile Phase: Acetonitrile:10mM Ammonia Acetate in Water (95:5)
Flow Rate: 0.2mL/min
Detection: UV@240nm
Column Temperature: 30ºC
Injection: 50µg/mL x 0.5µL

9 injections in parallel shows good reproducibility



UHPLC Column: VisionHT™ HILIC, 1.5µm, 2 x 50mm (Part No. **5141919**)
Mobile Phase: Acetonitrile:Water(20mM Ammonium Formate) (90:10)
Flow Rate: 0.2mL/min
Detection: UV@240nm
Column Temperature: 30ºC
Injection: 50µg/mL x 0.1µL

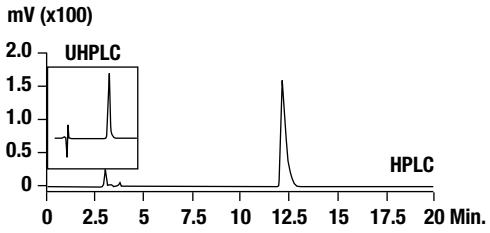


Conc.	Inj. (µL)	Weight (ng)	Peak Area
50µg/mL	0.1	5	7.3
50µg/mL	0.5	25	32
50µg/mL	1	50	63
50µg/mL	5	250	318
50µg/mL	10	500	641
50µg/mL	20	1000	1293

This method exhibits excellent linear response between 5ng and 1000ng for accurate quantitation.

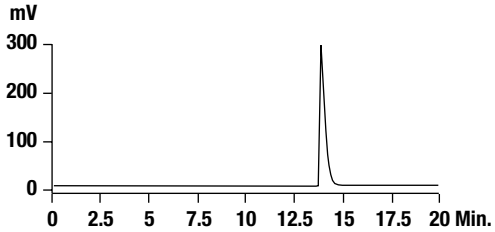
HPLC Method Transfer to UHPLC

The recent adoption of Fast LC systems within the laboratory environment means there is a period of time when most labs have a variety of LC systems types – Traditional, UHPLC, and/or alternate Fast LC systems. Optimizing and transferring methods between systems has not been a simple and intuitive task. However, when the identical bonded phase is available in sub2, 3, 5, and 10µm particle sizes, it can be applied to appropriate formats to suit the system type. A simple calculation to determine equivalent linear velocity is all that's necessary to seamlessly transfer methods between systems, or to optimize a method on one system and apply to an entirely different type of system. Compared to a conventional HPLC method, the UHPLC method is 4 times faster.



	HILIC Column	Flow Rate	Time (min)	Conc.	Inj. Range	Loading Range
HPLC	VisionHT™ HILIC, 5µm, 4.6 x 250mmL	1.0mL/min	12.090	40µg/mL	1-100µL	40ng-4mg
UHPLC	VisionHT™ HILIC, 1.5µm, 2.0 x 50mmL	0.2mL/min	3.259	50µg/mL	0.1-20µL	5ng-100ng

ELSD Methods for Melamine



HPLC Column: VisionHT™ HILIC, 5µm, 4.6 x 250mm (Part No. **86466**)
Detector: Alltech® 3300 ELSD (Part No. **5135834**)
Mobile Phase: Acetonitrile:10mM Ammonium Acetate in Water (95:5)
Flow Rate: 1mL/min
Detection: UV@240nm
Column Temperature: 30ºC
ELSD 3300 Settings: drift tube 40ºC, gas 1.8L/min, gain x 4
Injection: 40µg/mL x 20µL

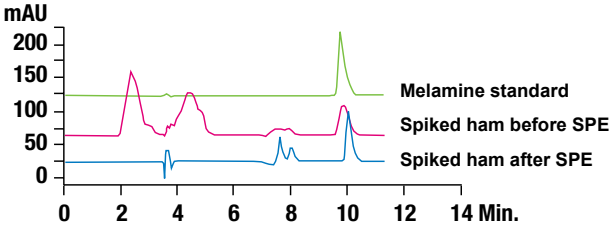
SPE Methods for Melamine

Extracting melamine from various food matrices using solid phase extraction is often required to obtain accurate quantitation. GracePure™ Cation-X, a strong cation exchange resin delivers a cleaner, more concentrated sample.

Melamine from Ham

SPE Column Type: GracePure™ SPE Cation-X, 500mg/3mL (Part No. **5138770**)
Sample Pretreatment: weight ham 2g
mix with 20mL 1% Trichloroacetic Acid and 5mL Acetonitrile
Sonicate for 30mins
Centrifuge for 15mins
Filter the supernatant with 0.45µm syringe filter, then spike with melamine standard solution

SPE Steps:
Column Conditioning: 2 column volumes methanol, then 2 column volumes water
Sample Application: slowly apply 10mL ham extract through the column
Column Washing: 3 column volumes water, then 2 column volumes methanol
Eluent: 4mL Methanol:0.5M Ammonium Hydroxide (1V:1V)



SPE recovery*=92%

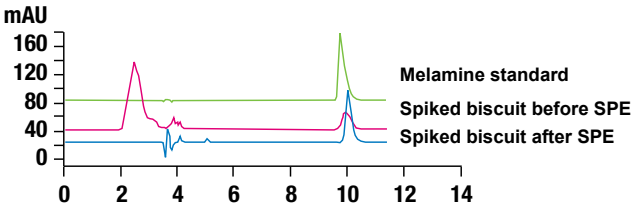
Mobile Phase: 10mM Ammonium Acetate:Acetonitrile (5:95)
HPLC Column: VisionHT™ HILIC, 5µm, 4.6 x 250mm
Detector: UV @ 240nm
Flow Rate: 1mL/min
Column Temperature: 30ºC
Injection: 10µL

SPE Methods for Melamine continued

Melamine from Biscuit

SPE Column Type: GracePure™ SPE Cation-X, 500mg/3mL, (Part No. **5138770**)
Sample Pretreatment: weight biscuit 2g
mix with 20mL 1% Trichloroacetic Acid and 5mL Acetonitrile
Sonicate for 30mins
Centrifuge for 15mins
Filter the supernatant with 0.45µm syringe filter, then spike with melamine standard solution

SPE Steps:
Column Conditioning: 2 column volumes methanol, then 2 column volumes water
Sample Application: slowly apply 10mL biscuit extract through the column
Column Washing: 3 column volumes water, then 2 column volumes methanol
Eluent: 4mL Methanol:0.5M Ammonium Hydroxide (1V:1V)



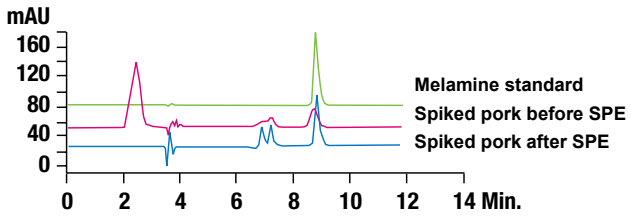
SPE recovery*=80%

Mobile Phase: 10mM Ammonium Acetate:Acetonitrile (5:95)
Column: VisionHT™ HILIC, 5µm, 4.6 x 250mm
Detector: UV @ 240nm
Flow Rate: 1mL/min
Column Temperature: 30ºC
Injection: 10µL

Melamine from Pork

SPE Column Type: GracePure™ SPE Cation-X, 500mg/3mL (Part No. **5138770**)
Sample Pretreatment: weight pork 2g
mix with 20mL 1% Trichloroacetic Acid and 5mL Acetonitrile
Sonicate for 30mins
Centrifuge for 15mins
Filter the supernatant with 0.45µm syringe filter, then spike with melamine standard solution

SPE Steps:
Column Conditioning: 2 column volumes methanol, then 2 column volumes water
Sample Application: slowly apply 10mL pork extract through the column
Column Washing: 3 column volumes water, then 2 column volumes methanol
Eluent: 4mL Methanol:0.5M Ammonium Hydroxide (1V:1V)



SPE recovery*=103%

HPLC Mobile Phase: 10mM Ammonium Acetate:Acetonitrile (5:95)
Column: VisionHT™ HILIC, 5µm, 4.6 x 250mm
Detector: UV @ 240nm
Flow Rate: 1mL/min
Column Temperature: 30ºC
Injection: 10µL

Conclusions

Fast, isocratic UHPC and HPLC methods were developed for the determination of melamine. Linearity range for melamine is 40 - 4000 ng. Since the Grace® VisionHT™ HILIC UHPLC and the HILIC Analytical HPLC columns are based on the same media, methods developed on the HPLC column can be easily transferred to UHPLC and vice versa. Compared to the HPLC method, UHPLC is 4 times faster. Mobile phases used in these methods are MS and ELSD compatible.

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