

Analysis of Melamine and Metformin Hydrochloride by HPLC-UV

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Key Words

- BioBasic SCX
- Melamine
- Metformin
- Clinical
- USP

Abstract

The United States Pharmacopeia (USP) monograph for the analysis of metformin stipulates that an L9 column must be used. Thermo Scientific BioBasic SCX fulfills the “strongly acidic cation exchanger” requirement for the L9 packing and, when used for the metformin method, achieves the system suitability criteria specified in the monograph.

Introduction

Melamine has many uses in both the industrial and agricultural fields. Mixed with resins melamine exhibits fire retardant properties and is frequently used to produce cooking utensils and fabrics. Melamine contains 66% nitrogen by mass and can be used in crop fertilization, however due to elevated costs in comparison to other fertilizers it is economically impractical. Due to the high nitrogen content, melamine has in the past been used in China as an adulterant for feedstock and milk. The addition of nitrogen-rich melamine increases the apparent protein content of poor quality food. Exposure to melamine can result in kidney damage, renal failure and subsequently death.

Metformin is primarily used in the treatment of type 2 diabetes and insulin resistant conditions such as polycystic ovary syndrome (PCOS). Similarly to melamine, metformin has a polar, nitrogen-rich structure, hence the two compounds are regularly detected within the same chromatographic method.

Based on sulfonic acid chemistry, BioBasic™ SCX is a strong cation exchange phase which lends itself to the separation of melamine and metformin according to the USP monograph for metformin hydrochloride. We have demonstrated that this column successfully separates melamine and metformin, satisfying the USP criterion that the resolution is >10.



Experimental Details

Chemicals and Reagents	Part Number
Fisher Scientific buffer: Ammonium dihydrogen phosphate	A685-500
Fisher Scientific HPLC grade water	W/0106/17
Sigma Aldrich metformin	D5035
Sigma Aldrich melamine	M2659

Sample Handling Equipment

Liquid handling hardware: Thermo Scientific FinnPipette (100-1000 µL)	642090
Vials and closures: Thermo Scientific Premium Vial	60180-600

Separation Conditions

Instrumentation:	Thermo Scientific Surveyor Plus with PDA detector	
Column:	BioBasic SCX 5 µm, 250 x 4.6 mm	73205-254630
Mobile phase:	1.7% ammonium dihydrogen phosphate pH 3.0	
Flow rate:	1.0 mL/min	
Run time:	16 minutes	
Column temperature:	40°C	
Injection wash solvent:	Water	
UV detector wavelength:	218 nm	

Preparation of mobile phase: 17 g of ammonium dihydrogen phosphate was dissolved into 1 L of HPLC grade water. The pH was adjusted to 3.0 using phosphoric acid.

Solutions

Samples were prepared according to the USP specification:

The working standard contained 5 µg/mL of metformin and 2 µg/mL of melamine in mobile phase.

Data processing

Software:	Thermo Scientific ChromQuest 5.0 software
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Results

When separating melamine and metformin with BioBasic SCX using the method specified in the USP monograph, better peak tailing factors were achieved when the column was temperature controlled (Table 1). Note that there is no specification with regards to tailing factor in the USP monograph.

The resolution value achieved with BioBasic SCX was 26, which comfortably exceeded the value of 10, specified in the monograph.

Replicate injections of the melamine/metformin system suitability mix showed that BioBasic SCX produced reproducible retention and peak shape (Table 2 and Figure 1).

Temperature	Tailing factor, T_f	
	Peak 1: Melamine	Peak 2: Metformin
22°C (ambient)	1.1	1.5
40 °C	1.1	1.3

Table 1: The effect of temperature on peak shape

Results	Peak 1: Melamine	Peak 2: Metformin
Retention time, t_R /min	4.38	10.08
%RSD t_R	0.22	0.45
%RSD Area	0.33	0.78

Table 2: Statistical assessment based upon data derived from six replicate injections at 40°C

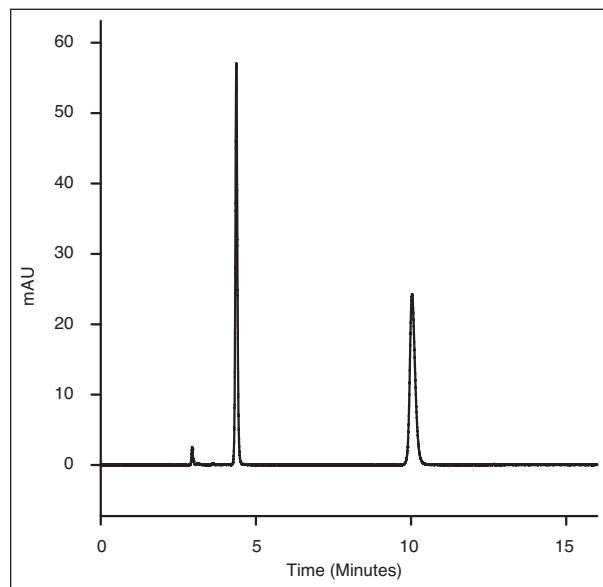


Figure 1: Separation of melamine and metformin using BioBasic SCX with a column temperature of 40°C

Conclusions

- BioBasic SCX can be used to successfully resolve melamine and metformin in accordance with USP system suitability criteria.
- Better peaks shapes and good retention time stability was achieved when the column was thermostatically controlled (Figure 1).

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