

# Analysis of Azo Dyes Using a Core Enhanced Technology Accucore HPLC Column

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## Key Words

- Accucore RP-MS
- Fused core
- Superficially porous
- Core Enhanced Technology
- Azo dyes

## Abstract

This application note demonstrates the use of the Thermo Scientific Accucore RP-MS HPLC column for the faster analysis of Azo Dyes.

## Introduction

Accucore™ HPLC columns use Core Enhanced Technology to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimised phase bonding creates a series of high coverage, robust phases. Accucore RP-MS uses an optimized alkyl chain length for more effective coverage of the silica surface. This coverage results in a significant reduction in secondary interactions and thus highly efficient peaks with very low tailing. The tightly controlled 2.6 µm diameter of Accucore particles provides much lower backpressures than typically seen with sub-2 µm materials.

Azo compounds are compounds with the functional group R-N=NR' where R,R' can be alkyl or aryl. The aryl azo compounds undergo n-delocalisation producing vivid colors such as reds, oranges and yellows. These are commonly referred to as azo dyes and are used as food coloring agents. In the food industry these are more commonly known as E numbers. Some of these dyes have been banned due to the toxicity of their degradation products which have been found to be mutagens and carcinogens.

The Accucore RP-MS shows good resolution, faster analysis time and an alternate selectivity in the analysis of azo dyes enabling high sample throughput.



## Sample Preparation

Individual 1 mg/ml standards of Fast Garnet, Orange II, Dimethyl Yellow, Sudan Red G, Sudan I, Sudan III and Sudan IV were made in DMSO.

The working standard mix contained:  
600 µl of Fast Garnet, 100 µl of Orange II, 150 µl of Dimethyl Yellow and 50 µl of Sudan Red G, Sudan I, Sudan III and Sudan IV.

Thermo Scientific Column	Part Number
Accucore RP-MS 2.6 µm 150 x 4.6 mm	17626-154630
Measured pressure: 300 bar	

Thermo Scientific Accela	
Column temperature	50 °C
Injection volume	2.0 µL
Flow rate	1.8 mL/min
UV detection	225 nm

## Mobile Phase

Mobile phase A: 0.1% TFA in Water

Mobile phase B: 0.1% TFA in Acetonitrile

Gradient: 0 min 25% B. 3 min 30% B. 15 min 95% B. 17 min 95% B. 17.01 min 25% B. 20 min 25% B.

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17
Fisher Scientific HPLC grade Trifluoroacetic acid	T/3258/PB05
Fisher Scientific Analytical Grade Dimethyl sulfoxide	D/4121/PB08
NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

## Results

The analysis was carried out on an Accucore RP-MS 2.6 µm 150 x 4.6 mm column. As shown in Figure 1, the separation of Fast Garnet, Orange II, Dimethyl Yellow, Sudan Red G, Sudan I, Sudan III and Sudan IV can be achieved in approximately 15 minutes. Accucore RP-MS provides the optimum separation of Azo dyes.

## Conclusions

The use of an Accucore RP-MS column for the analysis of azo dyes successfully separates the dyes in 15 minutes, producing good peak shape and resolution. Accucore RP-MS columns are therefore an excellent choice for the fast analysis of azo dyes, allowing high sample throughput.

Peak	Analyte	Asymmetry (average)	Retention Time (average)	Retention Time (% RSD)
1	Fast Garnet	1.60	3.42	0.47
2	Orange II	1.42	3.60	0.40
3	Dimethyl Yellow	1.09	7.94	0.96
4	Sudan Red G	1.09	10.77	0.07
5	Sudan I	1.07	10.98	0.07
6	Sudan III	1.08	13.48	0.05
7	Sudan IV	1.07	14.76	0.04

Table 1: Peak Identity, asymmetry and resolution (based on the average of 6 replicate injections) of the azo dyes as obtained on the Accucore RP-MS

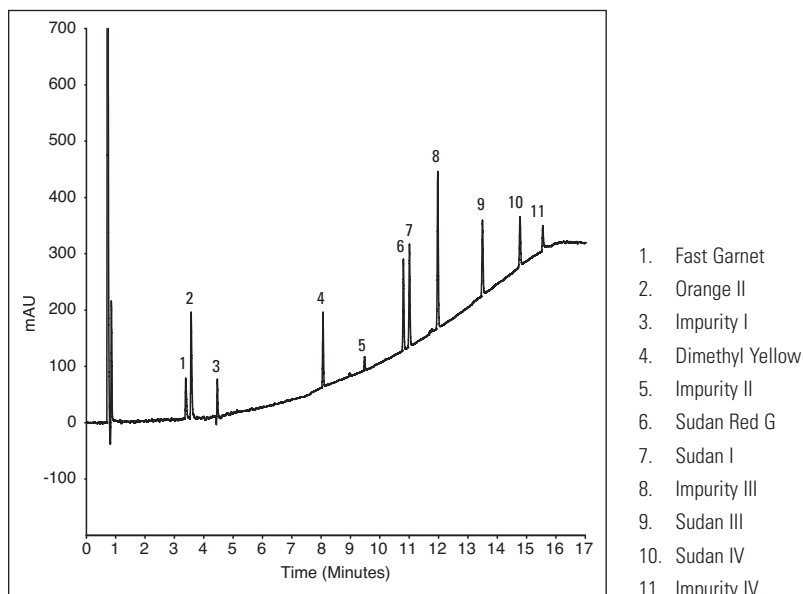


Figure 1: Chromatograph of the azo dyes separated on Accucore RP-MS 2.6 µm 150 x 4.6 mm column

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