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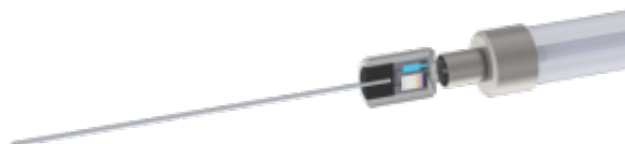
## $\mu$ SPEed | Application Note 2015

### $\mu$ SPEed recovery curve with 4 nitrotoluene

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#### INTRODUCTION

The aim was to prepare a five point calibration curve from a 1000 ppm stock solution of 4-nitrotoluene, dilute the standards by 1:2, and then use SPE to concentrate the diluted standards back to original concentration.



#### PROCEDURE

##### $\mu$ SPEed Extraction Process

1. Dilution Step 1:  
From a 1000 ppm standard of 4-nitrotoluene, 1 mL standards were prepared at 10, 20, 30, 40, 50 ppm with water. The 1000  $\mu$ L syringe was used to transfer 900  $\mu$ L of water into five vials. The 100  $\mu$ L syringe was used to transfer, 90, 80, 70, 60 and 50  $\mu$ L of water into the five vials. The 100  $\mu$ L syringe was then used to transfer 10, 20, 30, 40, 50  $\mu$ L of the 1000 ppm 4-nitrotoluene standard.
2. Dilution Step 2:  
The standards from dilution step 1 were diluted 1:2. The 1000  $\mu$ L syringe was used to transfer 500  $\mu$ L of water into another set of five vials, and 500  $\mu$ L of the standards in step 1 were dispensed into their respective vial.
3. SPE using  $\mu$ SPEed cartridges:  
Standards obtained at dilution step 2 were loaded onto C18  $\mu$ SPEed cartridges (200  $\mu$ L) and eluted with MeOH (100  $\mu$ L). The  $\mu$ SPEed workflow was:
  - a. Condition the  $\mu$ SPEed cartridges with 200  $\mu$ L of methanol
  - b. Wash the cartridge with 200  $\mu$ L of water
  - c. Load the cartridge with the 200  $\mu$ L of sample
  - d. Wash the cartridge with 200  $\mu$ L of water
  - e. Elute the sample into a vial with 100  $\mu$ L of methanol

Table 1: Expected concentration for 4-nitrotoluene in each standard

Dilution step 1 (ppm)	Dilution step 2 (ppm)	SPE
10	5	10
20	10	20
30	15	30
40	20	40
50	25	50

### Chromatography

A Thermofisher Vanquish UHPLC system with UV detection was used for analysis, and the operating parameters shown in Table 2.

Table 2: UHPLC operating parameters for analysis of 4-nitrotoluene.

Separation conditions	
Column	C18+ column (100 mm x 2.1 mm, 1.5 $\mu$ m)
Flow rate	0.300 mL/min
Mobile phase composition	Isocratic method using 60:40 methanol to water
Injection volume	1 $\mu$ L
Run time	5 min
Column Temperature	35°C
UV detection	254 nm

### RESULTS AND DISCUSSION

The standards from dilution step 1 were used to create a five point calibration curve which showed good linearity ( $R^2$  0.9994) (see Figure 1).

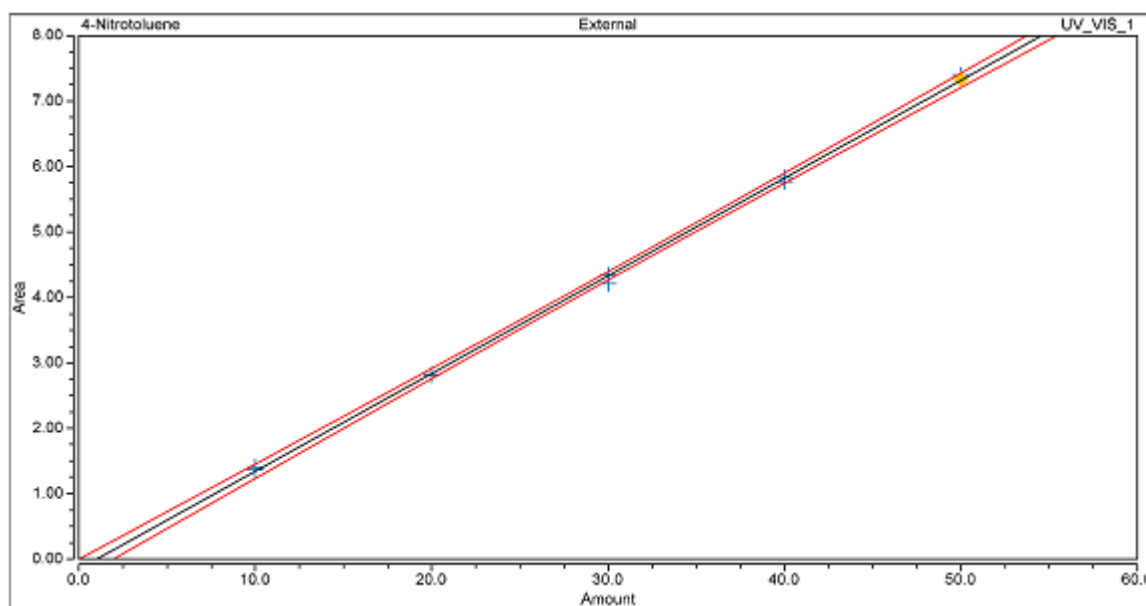


Figure 1: Five point calibration for 4-nitrotoluene with a linear regression of 0.9994.

The concentrations for standards obtained in dilution step 2 were determined from the calibration curve and the recovery calculated against the expected value (see Table 3). The two injections for standard 10/2 fell below the calibration range and thus showed a higher percentage recovery of 112%. However, the remaining standards that were within the calibration range showed good recoveries ranging from 101-110%.

Table 3: The determined concentration and percentage recovery of 4-nitrotoluene for dilution step 2 standards.

Injection	Amount (ppm)	Percentage recovery from the expected concentration (%)
10/2 (1)	5.6	112
10/2 (2)	5.6	112
20/2 (1)	11.7	110
20/2 (2)	10.8	108
30/2 (1)	16.1	108
30/2 (2)	15.9	106
40/2 (1)	20.9	104
40/2 (2)	21.3	106
50/2 (1)	25.2	101
50/2 (2)	25.2	101

Using the calibration curve, the concentration of 4-nitrotoluene was also determined following preconcentration of the diluted standards with SPE. The recoveries were calculated from the expected concentrations (see Table 4). The recoveries ranged from 104-107%, and therefore, showed good accuracy of the uSPEd cartridges.

Table 4: The determined concentration and percentage recovery of 4-nitrotoluene for the SPE standards.

Injection	Amount (ppm)	Percentage recovery from the expected concentration (%)
SPE 10 (1)	10.5	105
SPE 10 (2)	10.5	105
SPE 20 (1)	21.1	106
SPE 20 (2)	21.0	105
SPE 30 (1)	31.3	104
SPE 30 (2)	31.5	105
SPE 40 (1)	42.9	107
SPE 40 (2)	42.7	107
SPE 50 (1)	52.0	104
SPE 50 (2)	52.1	104

Figures 2 to 5 shows the chromatograms for standards 20, 20/2 and SPE 20



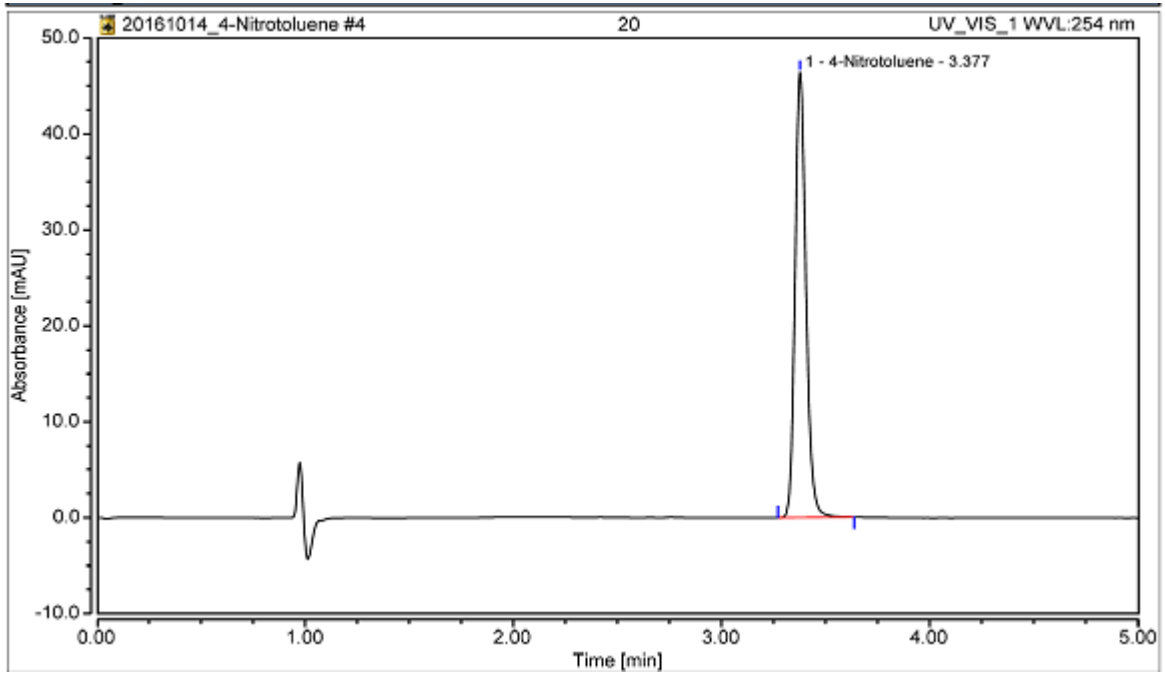


Figure 2: Chromatogram of 4-nitrotoluene in standard 20 (dilution step 2).

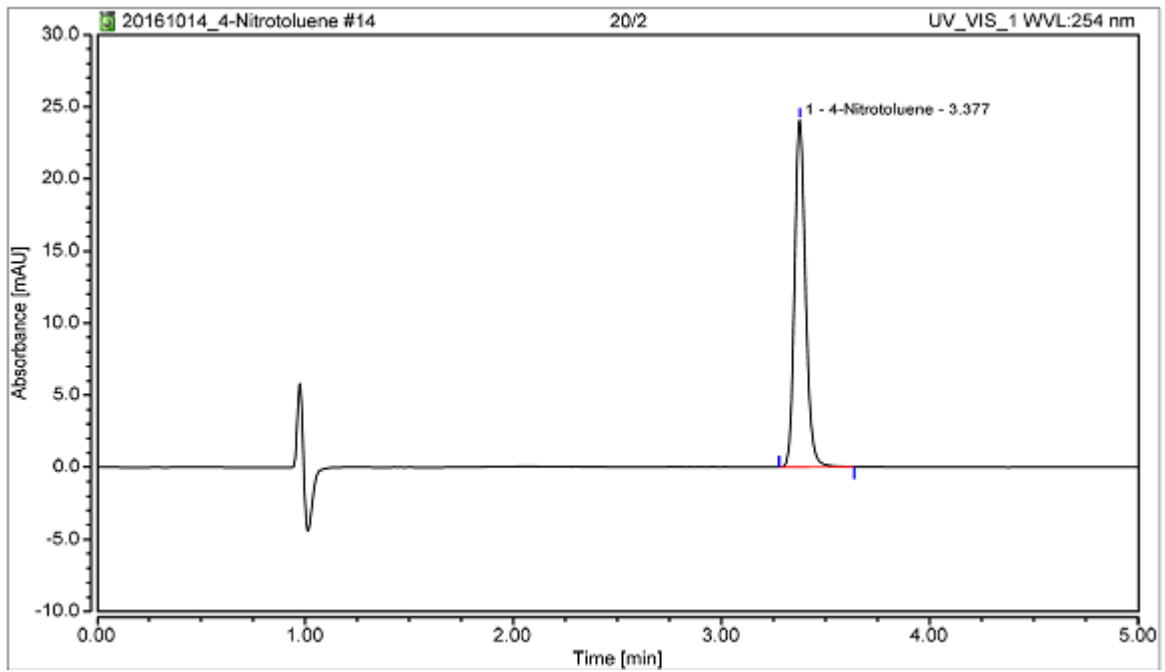


Figure 3: Chromatogram of 4-nitrotoluene in standard 20/2 (dilution step 2).



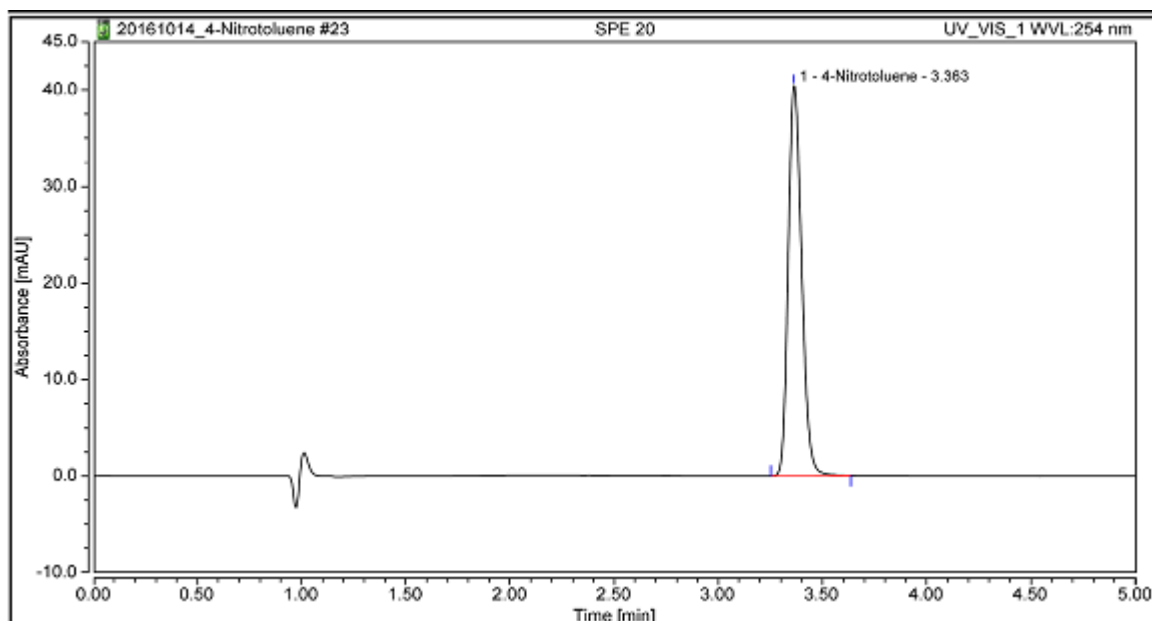


Figure 4: Chromatogram of 4-nitrotoluene in standard SPE 20.

## CONCLUSION

The ePrep automated sample preparation workstation provided accurate preparation and dilution of standards with acceptable percentage recoveries of 4-nitrotoluene ranging from 101-110%. The  $\mu$ SPEed C18 cartridges also showed good accuracy as with agreement between the reconcentrated samples performed by SPE and the original standards. The percentage recoveries were acceptable and ranged from 104-107%.

## ACKNOWLEDGEMENTS

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## REFERENCE

### $\mu$ SPEed Ordering Information

Part Number	Code	Description
<b><math>\mu</math>SPEed Cartridges</b>		
01-10110	$\mu$ SPEed, C18RPS-3 $\mu$ m/120Å (Pkt 10)	3 $\mu$ m/ 120Å ODS spherical silica packing with high acidic resistance suitable for general organic compound applications.
01-10115	$\mu$ SPEed, Silica-3 $\mu$ m/120Å (Pkt 10)	3 $\mu$ m/120Å spherical bare silica packing. High purity silica for normal and hilly applications
01-10150	$\mu$ SPEed, PS/DVB -3 $\mu$ m/ 300Å (Pkt 10)	3 $\mu$ m/ 300Å spherical, crosslinked polystyrene divinyl benzene
01-10151	$\mu$ SPEed, PS/DVB RP-3 $\mu$ m/ 300Å (Pkt 10)	3 $\mu$ m/ 300Å Phenyl (RP) spherical, crosslinked polystyrene divinyl benzene
01-10155N	$\mu$ SPEed, PS/DVB SAX-3 $\mu$ m/ NP (Pkt 10)	3 $\mu$ m/Non Porous SAX spherical, crosslinked polystyrene divinyl benzene
01-10156N	$\mu$ SPEed, PS/DVB SCX-3 $\mu$ m/ NP (Pkt 10)	3 $\mu$ m/Non Porous SCX spherical, crosslinked polystyrene divinyl benzene