

Novel Polymer-Based Stationary Phase for On-Line SPE-HPLC Analysis of Phenols

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ABSTRACT

The presence of phenols, commonly used pharmaceuticals, and pesticides in the water supply is of high interest and methods are in development to provide high-throughput screening using SPE-LC-MS/MS methodologies. The new Dionex solid-phase extraction (SPE) phase is a hydrophilic reversed-phase material comprised of porous divinylbenzene (DVB) resin grafted with hydrophilic polymers. Packed columns are designed for the online preconcentration of organic compounds with a wide range of hydrophilicity via adsorption, π - π interaction mechanisms. The hydrophilic character of DVB materials allows concentration of analytes from large volumes of water without evidence of de-wetting, improved retention of hydrophilic compounds, and better bed stability. The SPE phase has good pressure stability and is fully compatible with HPLC methods. The on-line SPE-HPLC front-end is coupled to ESI-MS and ESI-MS/MS. Typical applications include the determination of trace-level phenolic compounds, pharmaceuticals, and pesticides in water.

Here, the application of a new stationary phase to the determination of trace-level phenolic compounds in water is presented.

INSTRUMENTATION

Dionex UltiMate® 3000 HPLC system consisting of:

- DGP-3600M Dual Gradient Pump
- SRD-3600 Solvent Rack with Integrated Vacuum Degasser
- TCC-3200 Thermostatted Column Compartment with two 2-position, 6-port valves
- PDA-3000
- AS-HV High-Volume Autosampler

Mass spectrometer:

- Applied Biosystems API 2000™

Columns:

- SolEx™ HRP cartridge, 2.1 × 20 mm
- Acclaim® PolarAdvantage (PA) reversed-phase column, 2.1 × 150 mm (incorporates a polar sulfonamide group near the surface of the silica particle)

Chromeleon® Chromatography Data System software version 6.80

DCMS^{Link™} 2.8 for Analyst® software

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SolEx HRP Cartridge Specification

Column chemistry:	Hydrophilic divinylbenzene (graft of poly-(N-vinylpyrrolidone) polymer onto macroporous divinylbenzene resin)
Particle size:	12–14 μm
Resin amount:	150 mg
pH range:	0–14
Solvent compatibility:	0–100% acetonitrile
Flow rate range:	0.2–5 mL/min
Backpressure range:	0–600 bar



EPA METHODS

- Based on analytical feasibility, the national primary drinking water regulation established a maximum contaminant level (MCL) of 1 ppb for pentachlorophenol. The other 10 common phenols listed in EPA method 604 are also on the U.S. EPA priority pollutants list.
- Federal Register Vol. 72, No. 59/Monday, March 29, 2010 states that "EPA reviewed performance testing (PT) data from the first six-year review cycle and analyzed more recent PT data to determine if the practical quantitation limit (PQL) can be revised (i.e., analytical feasibility). A review of analytical feasibility did not identify a potential to revise the MCL, which is limited by feasibility."

Approved methods for the detection of pentachlorophenol include:

- EPA method 515.1: Determination of chlorinated acids in water by gas chromatography with an electron capture detector.
- EPA method 515.2: Determination of chlorinated acids in water using liquid-solid extraction and gas chromatography with electron capture detector.
- EPA method 525.2: Determination of organic compounds in drinking water by liquid-solid extraction and capillary gas chromatography/mass spectrometry.

Figure 1 is a schematic of the devices used for the determination of phenols using online SPE followed by HPLC with UV and ESI/MS/MS detection. The Dionex high-volume autosampler (AS-HV) has a peristaltic pump that can draw samples from the sample bottle in the pull mode to avoid contamination. The movement of the AS-HV is controlled by Chromeleon software. The AS-HV uses the left valve of TCC-3200 module as a sample valve and the right valve as an online SPE switching valve.

A fully automated SPE-LC analysis cycle using Dionex UltiMate 3000 $\times 2$ dual gradient HPLC systems consists of four distinct steps:

1. Matrix depletion and analyte extraction

(left valve 1_2; right valve 6_1)

The sample is injected onto the SPE column. Analytes are retained; matrix flushed to waste.

2. Analyte transfer from the SPE column to the analytical column

(left valve 1_2; right valve 1_2)

After switching the right valve, SPE and analytical columns are connected in line. The mobile phase delivered by the micropump desorbs the retained analytes from the SPE column in backflush mode and transfers analytes to the analytical column.

3. Analyte separation

(left valve 1_2; right valve 6_1)

Analytes are separated on the analytical column using a linear gradient mobile phase delivered by the micropump.

4. Washing and equilibration of the SPE and analytical column

(left valve 6_1; right valve 6_1)

To prevent carryover, the SPE and analytical column are washed and re-equilibrated.

Sample overlap injections: Rinse and sample loading to 10 mL sample loop for the following run starts after step 1. Washing and re-equilibration of SPE column overlaps with the analyte separation.

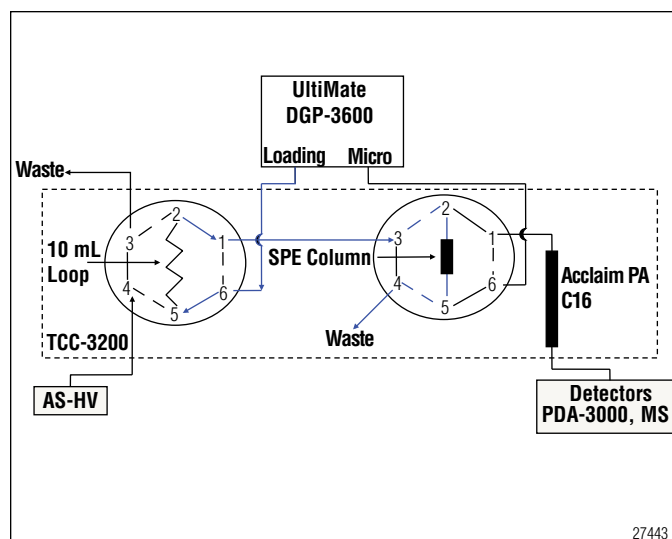
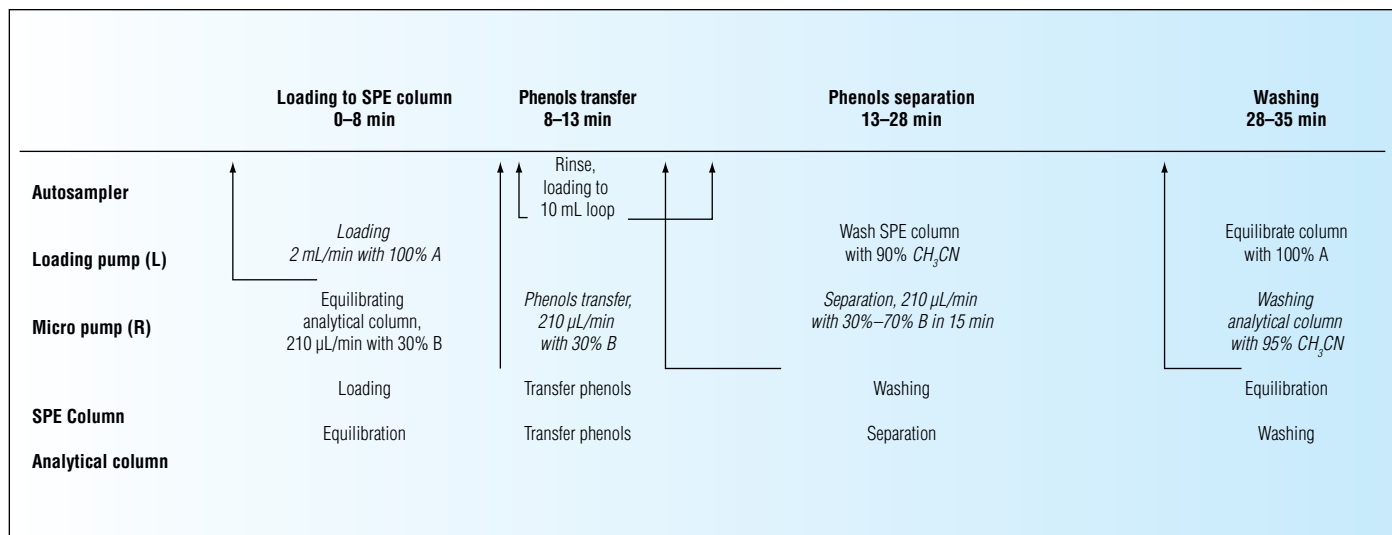


Figure 1. Schematic of devices for online SPE.

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Timeline diagram.

CONDITIONS

Solid-Phase Extraction

Concentrator: SolEx HRP cartridge, 2.1 × 20 mm
 Mobile phase for SPE:
 Loading pump: A. 0.02% HCOOH; B. CH₃CN
 Loading: 2 mL/min with 100% A for 8 min
 Washing of SPE column: 2 mL/min with 90% B

Separation

Analytical column: Acclaim PA C16, 3 µm, 2.1 × 150 mm
 Mobile phase for analysis:
 Micro pump: A. 25 mM HAC/NH₄Ac (1.45:1, v/v);
 B. CH₃CN
 Phenols transfer: 210 µL/min with 30% B for 5 min
 Phenols separation: 210 µL/min, 30% to 70% B in 15 min
 Washing of analytical column: 210 µL/min, 95% B for 7 min

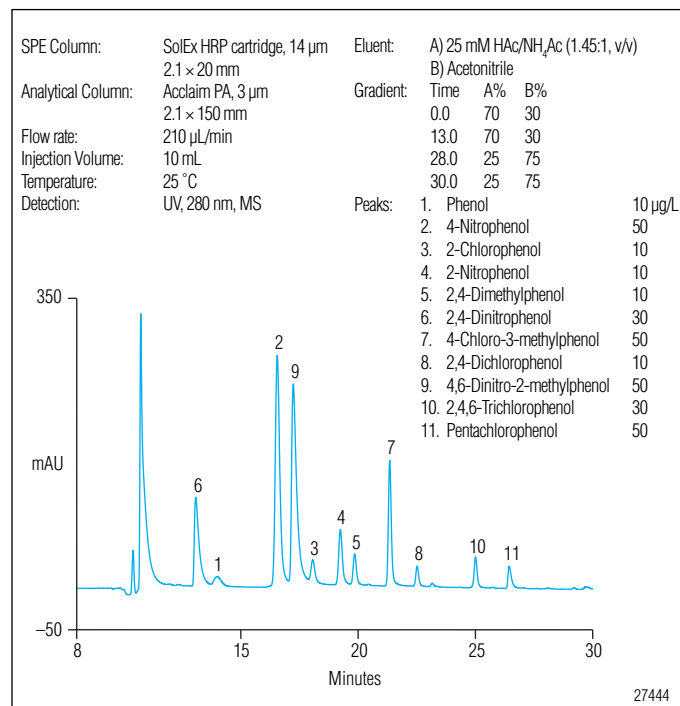


Figure 2. Online SPE and separation of phenols.

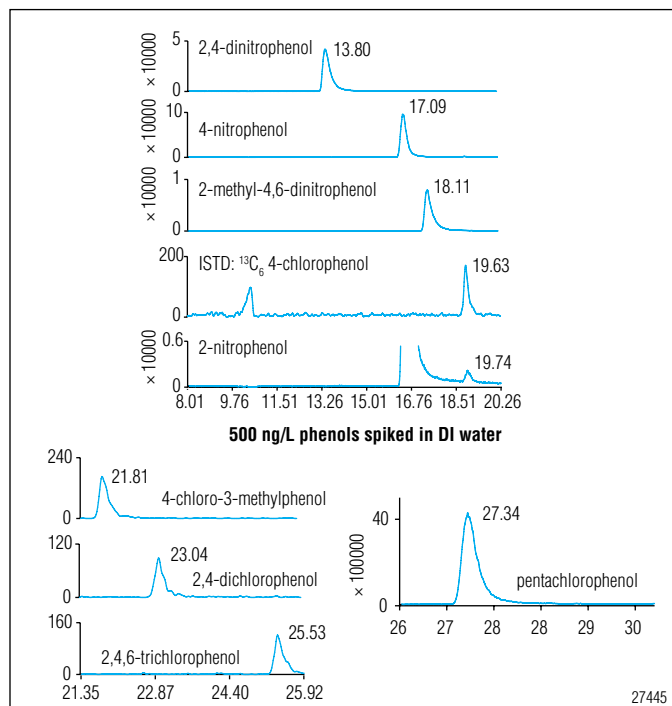


Figure 3. Overlay of negative ESI MRM results.

Table 1. Negative ESI MRM Method Parameters

Analytes	Retention Time (min)	Precursor Ion (m/z)	Product Ion (m/z)	Declustering Potential (volts)	Collision Energy (volts)
2,4-Dinitrophenol (24DNP)	13.91	183.0	109.1	-20	-33
4-Nitrophenol (4NP)	17.19	138.0	108.0	-15	-23
4,6-Dinitro-2-methylphenol (46DN2MP)	18.15	197.0	109.0	-19	-30
2-Nitrophenol (2NP)	19.70	183.0	109.1	-20	-21
ISTD, ¹³ C ₆ 4-chlorophenol	19.75	132.9	97.1	-35	-22
4-Chloro-3-methylphenol (4C3MP)	21.89	140.9	105.0	-40	-22
2,4-Dichlorophenol (24DCP)	23.10	161.0	89.0	-20	-21
2,4,6-Trichlorophenol (246TCP)	25.58	195.0	123.0	-18	-35
Pentachlorophenol (PCP)*	27.34	264.9			

*Pentachlorophenol detection using Q1 only.

Table 2. Method Detection Limits

Analytes	MDL in DI water (ng/L)
2,4-Dinitrophenol (24DNP)	6.7
Phenol	2330*
4-Nitrophenol (4NP)	0.12
4,6-Dinitro-2-methylphenol (46DN2MP)	3.1
2-Chlorophenol (2CP)	1150*
2-Nitrophenol (2NP)	28.7
2,4-Dimethylphenol (24DMP)	1160*
4-Chloro-3-methylphenol (4C3MP)	13.9
2,4-Dichlorophenol (24DCP)	14.8
2,4,6-Trichlorophenol (246TCP)	14.9
Pentachlorophenol (PCP)	0.93

*The MDLs of phenol, 2, 4-dimethylphenol, and 2-chlorophenol are calculated by UV detection data. MDLs of all other phenols are calculated by MS detection data.

Table 2 shows the method detection limit (MDL) from 6 replicate injections of the 11 measured phenols in DI water. The single-sided Student's *t* test (at the 99% confidence limit) was used for estimating MDL, where the standard deviation of the peak area of 6 injections is multiplied by Student's *t* value 3.365 (*n* = 6) to yield the MDL.

In Figure 4, the calibration plots on the left side are from ESI/MS/MS response and those on the right side are from the UV response. In general, ESI is much more sensitive than UV detection for charged or ionizable analytes. However, for analytes with high ESI-MS/MS response, like 4-nitrophenol (negative ion ESI, 1 pg injection, 300:1 S/N), the API 2000 spectrometer is capable of generating a linear response from 1 pg (1 ppt × 10 mL) to 1 ng (1 ppb × 10 mL). MS is saturated with amounts higher than 1 ng. It is necessary to use UV detection for higher amounts. Undissociated weakly acidic phenols, like phenol (pKa 9.95), 2, 4-dimethylphenol (pKa 10.6) or 2-chlorophenol (pKa 8.52), have very low sensitivity with ESI detection. Their quantitation range is determined by UV only.

4-Chlorophenol (¹³C₆, 99%) is used as the internal standard for ESI/MS/MS detection and its concentration is 0.25 ppb in all spiked samples.

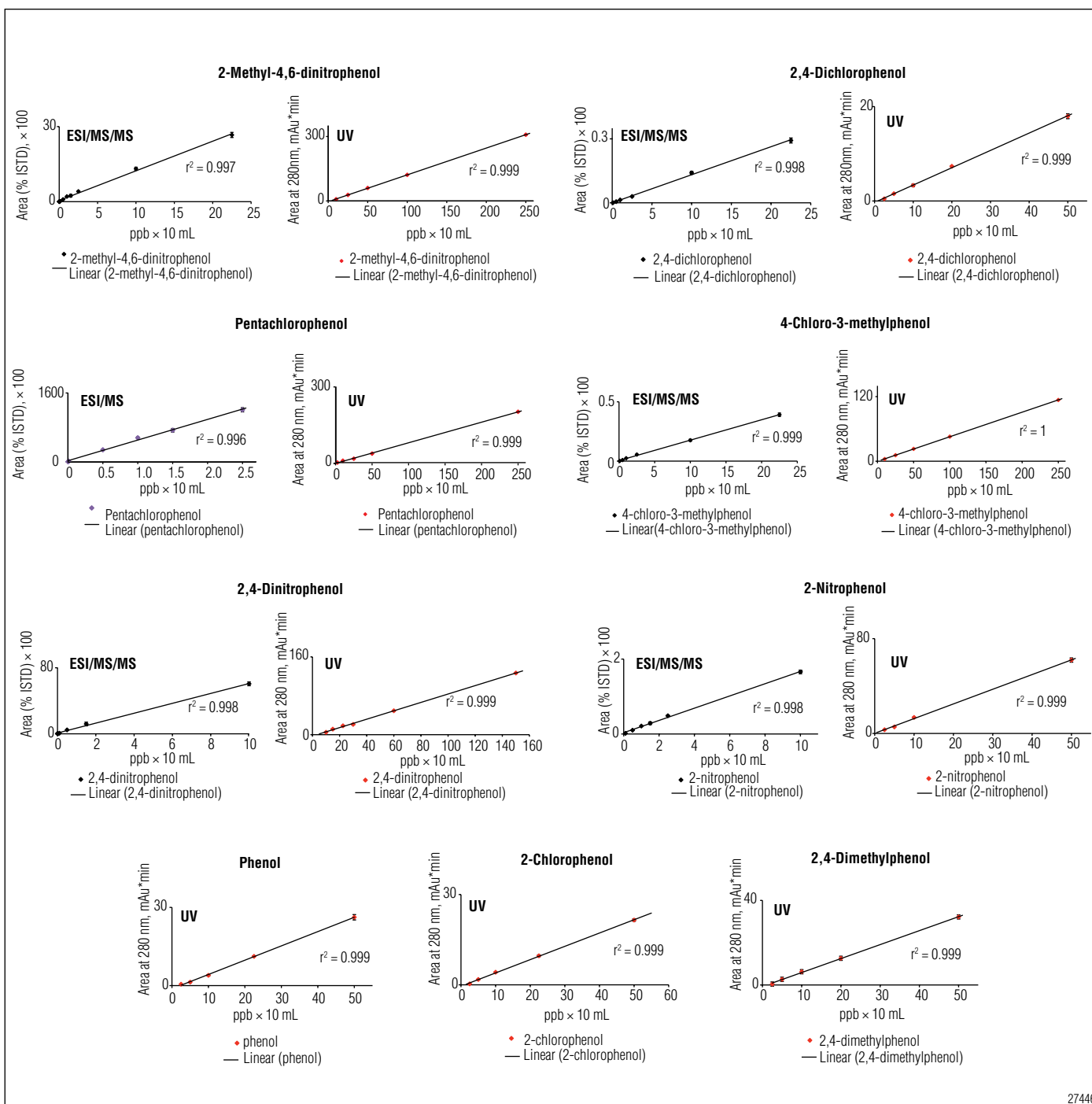


Figure 4. Calibration curves.

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Table 3. Recovery of Phenols in Sunnyvale Tap Water

Analytes	% Recovery		
	0.5 µg/L	10 µg/L	22.5 µg/L
	ESI-MS	ESI-MS, UV	UV
2,4-Dinitrophenol	88.3%	91.4% *	92.5%
Phenol	not detected	112.6%	86.5%
4-Nitrophenol	100.0%	96.6%	92.8%
2-Methyl-4,6-dinitrophenol	81.8%	110.0%*	98.0%
2-Chlorophenol	not detected	110.7%	102.8%
2-Nitrophenol	112.9%	102.7%	103.0%
2,4-Dimethylphenol	not detected	95.1%	94.3%
4-Chloro-3-methylphenol	92.8%	99.7%	96.2%
2,4-Dichlorophenol	111.7%	103.7%	95.1%
2,4,6-Trichlorophenol	92.1%	113.0%	96.3%
Pentachlorophenol	94.6%	98.7%	97.8%

*Determined by ESI-MS/MS.

Recovery % calculated by comparing the area responses of phenols spiked in Sunnyvale tap water to the same sample spiked in DI water.

CONCLUSION

- Using SPE and LC-UV-MS/MS, phenols in negative-ion mode were analyzed at low pico-gram to nano-gram level on the new Dionex SPE phase and the Acclaim PA analytical column. Good linearity was observed for analytes from 10 pg to 10 ng on column.
- Repeatability study from 6 injections of target analytes at 5 ng on column showed RSDs below 5%.
- The method was applied to Sunnyvale tap water sample extracts, that demonstrated good recovery ranging from 5 ng to 225 ng.
- The new Dionex SPE stationary method allows concentration of analytes from large volumes of water without evidence of de-wetting, and also provides improved retention of hydrophilic compounds, and better bed stability.

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