

Advantages in online SPE using the 8-port 2-position and multi-injection valve for PAH analysis

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SUMMARY

Solid phase extraction (SPE) is a useful technique to enhance detection limits to analyse even very small amounts of compounds. Most often SPE is carried out manually but coupled to an HPLC system, the so called online SPE technique allows an automated sample analysis. The used system configuration makes it possible to switch between direct injection via autosampler and injection with an auxiliary pump (aux/feed pump) without changing/replumbing the system.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants and are widespread across the globe mainly due to long-term anthropogenic sources of pollution [1]. Due to their high mutagenic and carcinogenic potential a sensitive determination is mandatory. SPE as a part of sample preparation is often necessary because of low quantification and detection limits that need to be achieved. Most often SPE is carried out manually but when coupled to an HPLC system, the so called online SPE technique allows an automated sample analysis and is a useful technique to enhance detection limits. Another advantage of SPE sample preparation in general lies in matrix reduction which is also beneficial for the limits of detection and quantification.

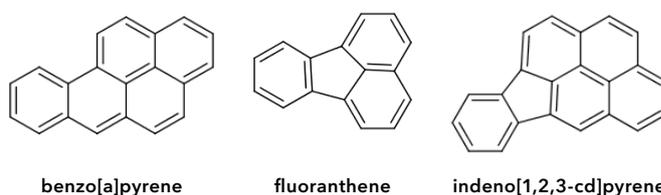


Fig. 1 Exemplary structures of selected PAHs

With the different switching possibilities of the configured valves, it is possible to manually load a sample, to use an autosampler or to use the auxiliary pump for a larger sample volume without replumbing the system.

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SAMPLE PREPARATION

EPA 610 PAH mixed standard (Sigma Aldrich, product number: 4S8743) came as a ready to use solution. The mixed standard was diluted with acetonitrile:water 60:40 (v/v) to different concentrations.

RESULTS

The method for determination of PAHs described in application note VEV0088 uses a configuration with three different valves. On basis of this application and hardware setup the different switching possibilities will be explained.

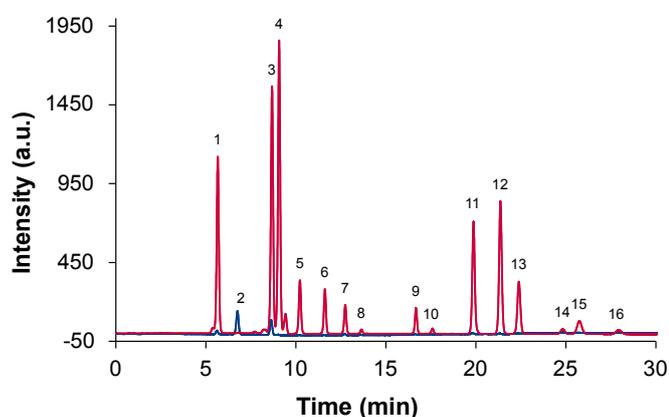


Fig. 2 Exemplary chromatogram of 16 PAH according EPA method 610 [2], red - fluorescence trace, blue - UV trace 230 nm, (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benzo[a]anthracene, (10) chrysene, (11) benzo[b]fluoranthene, (12) benzo[k]fluoranthene, (13) benzo[a]pyrene, (14) dibenzo[a,h]anthracene, (15) benzo[g,h,i]perylene, (16) indeno[1,2,3-cd]pyrene

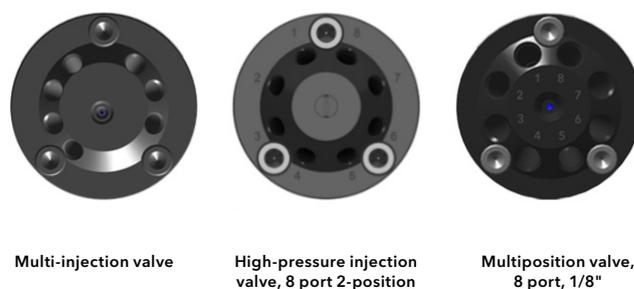


Fig. 3 Used valves in system configuration

With the valve settings displayed in **Fig. 4**, the main column is operated with the binary gradient pump and an injection via autosampler is possible. Theoretically, the SPE cartridge can be loaded manually from the syringe position of the multi-injection valve. Due to the used particle size of the SPE column and the resulting backpressure, loading the SPE with a syringe can be difficult to implement.

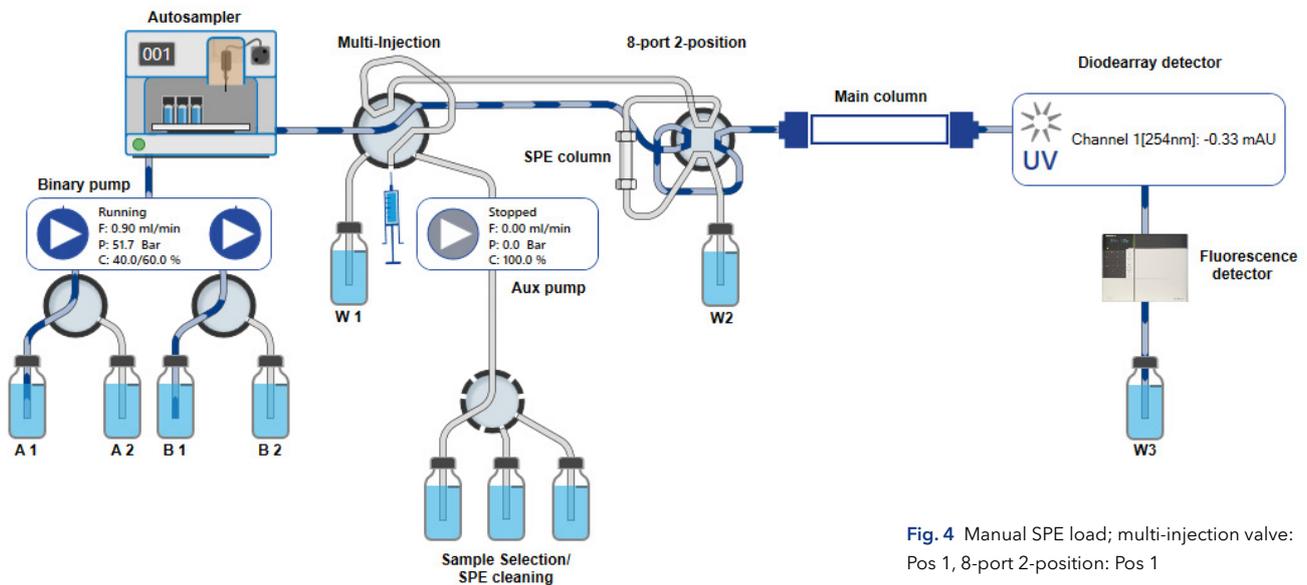


Fig. 4 Manual SPE load; multi-injection valve: Pos 1, 8-port 2-position: Pos 1

When switching the 8-port 2-position valve to position 2 (Fig. 5) the SPE is now in the flow path of the binary pump and the manually loaded sample will be eluted onto the main column for analysis. When using position 1 of the multi-injection valve, no automated cleaning of the SPE cartridge is possible because it is not connected to the aux pump.

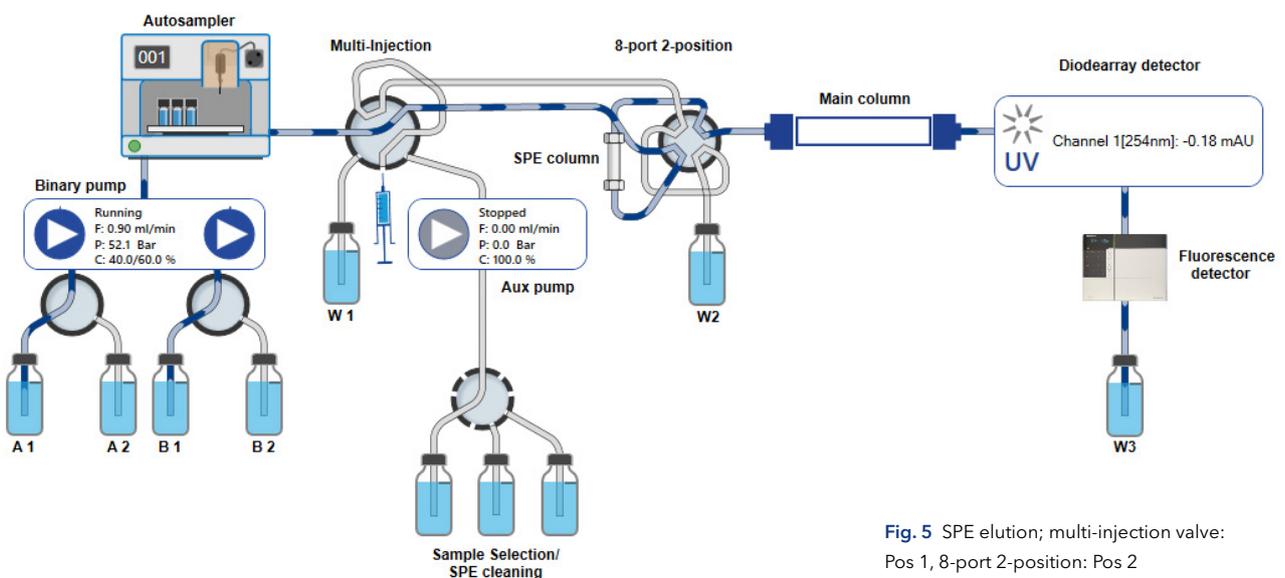


Fig. 5 SPE elution; multi-injection valve: Pos 1, 8-port 2-position: Pos 2

Direct injection using the autosampler is also possible with the following setting: Multi-injection valve: 2, 8-port 2-position: 1. The SPE cartridge cannot be loaded with these conditions. Also, the aux pump would be directly connected to the waste and has no functionalities. With the setting: multi-injection valve: 2, 8-port 2-position: 2, the SPE column is switched into the flow path but because it couldn't be loaded before, these settings were not usable for the application and are therefore not displayed.

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In **Fig. 6**, the sample is injected onto the SPE column via the autosampler. Dependent on the used syringe and sample loop, different volumes can be loaded. Switching the 8-port 2-position valve to position 2, will result in a connection of the binary pump to waste (W2).

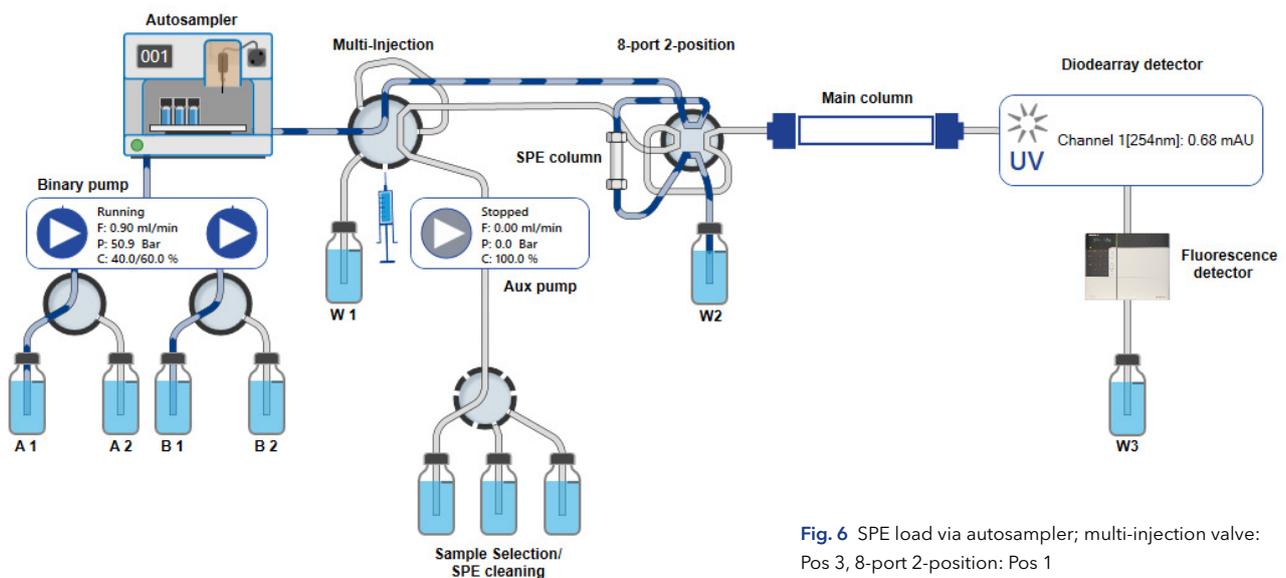


Fig. 6 SPE load via autosampler; multi-injection valve: Pos 3, 8-port 2-position: Pos 1

The settings shown in **Fig. 7** were used as starting conditions for the PAH application. The binary pump is connected to the main column. The aux pump is connected to the SPE column and can perform automated conditioning, sample loading and cleaning with the solutions from the multiposition valve.

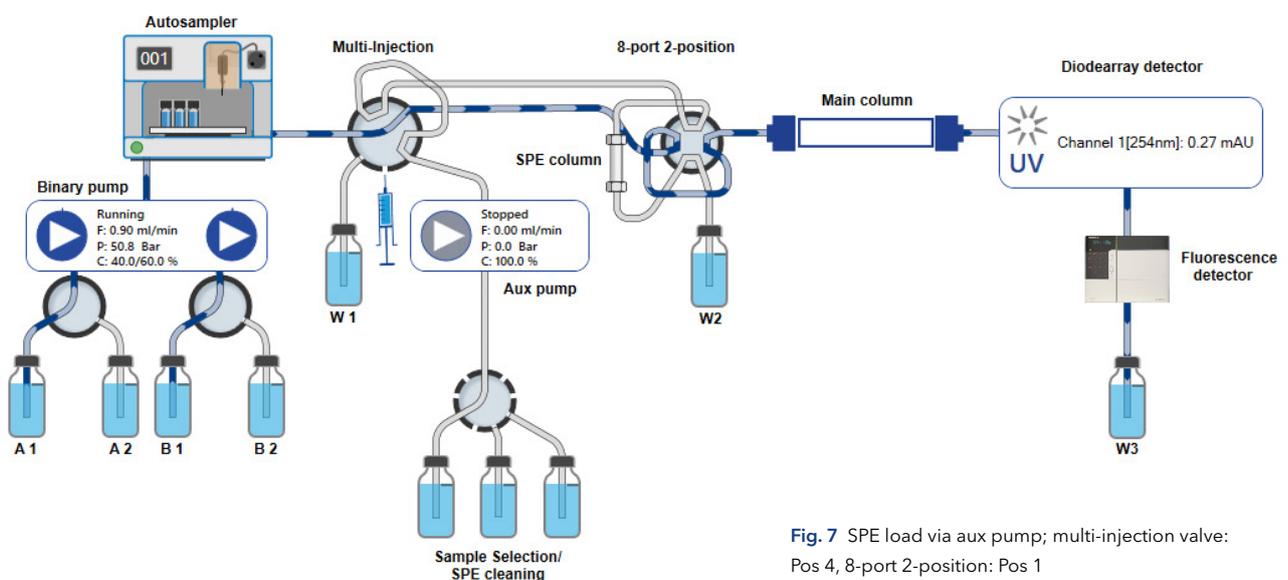


Fig. 7 SPE load via aux pump; multi-injection valve: Pos 4, 8-port 2-position: Pos 1

Switching the 8-port 2-position valve to position 2 (**Fig. 8**), the SPE is in the flow path of the binary pump, and the loaded sample will be eluted onto the main column for analysis.

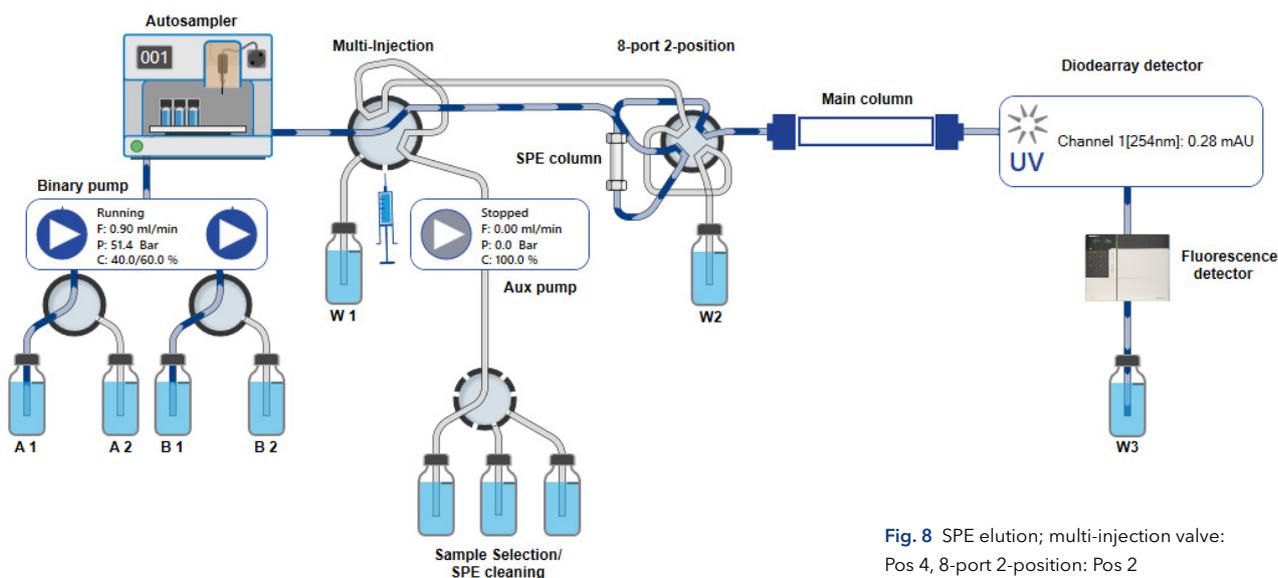


Fig. 8 SPE elution; multi-injection valve: Pos 4, 8-port 2-position: Pos 2

The following tables display the switching positions and aux pump flow rates that were used for the method in application note VEV0088 for loading the SPE via the autosampler.

Tab. 1 Valve switching program, SPE loading/elution/cleaning

Time (min)	Multi-injection valve	8-port 2-position valve	Multiposition valve 8port, 1/8"	Comment
0.00	4	1	1*	-
0.02	3	1	1	SPE load
0.20	4	1	1	SPE wash
1.00	4	2	1	SPE elution
30.00	4	1	1	SPE cleaning and conditioning
39.00	4	1	2**	
42.00	4	1	1	

* H₂O, ** acetonitrile

Tab. 2 Pump program for ASM 2.2L (aux pump), SPE washing/cleaning/conditioning

Time	Flow rate (ml/min)
0.00	0.00
0.20	0.00
0.22	3.00
1.00	3.00
1.02-39.00	0.00
39.02-44.00	3.00
45.00	0.00

CONCLUSION

The multi-injection valve enables three different SPE loading options: manually with syringe, via autosampler, and via feed pump. That makes the method very versatile and allows the user to inject a broad variety of different sample volumes from µl to ml scale. Referring to application note VEV0088, it was also demonstrated that loading higher sample volumes on the SPE cartridge will lead to an enhancement of the method sensitivity. The developed application shows that using special types of valves is both beneficial in terms of automation, time savings in sample preparation and detection limits.

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MATERIAL AND METHODS

System configuration

Component	Description	Article No.
Pump	AZURA P 6.1L HPG, 10 ml, 862 bar	APH35EA
Autosampler	AZURA AS 6.1L, Standard	AAA50AA
Detector UV	AZURA DAD 2.1L	ADC01
Flow cell	High Sensitivity KNAUER LightGuide UV Flow Cell Cartridge	AMD59XA
Detector FLD	Shimadzu RF-20A	A59200
Thermostat	AZURA CT 2.1	ATC00
Valve	AZURA Valve Unifier VU 4.1	AWA01XA
Valve	Multiposition Valve, 8 port, 1/8"	AVT34AE
Assistant	AZURA Assistant ASM 2.2L	AY03231
	Left module: Pump P 4.1S, 10 ml, stainless steel	DPG22EA
	Middle module: Valve drive VU 4.1	EWA04
	Right module: Valve drive VU 4.1	EWA04
Valve	High-pressure injection Valve, 8 Port 2-position	AVC38AC
Valve	Multi-injection Valve, DLC stainless steel	AVN96AE
Column	NUCLEOSIL 100-5 C18 PAH, 5 µm, 150×4 mm	
SPE column	Eurosil Bioselect 300-5 C8. 30×4 mm	03DK081EJ
Software	ClarityChrom 8.5 - Workstation, autosampler control included	A1670
Software	ClarityChrom 8.5 - PDA extension	A1676

REFERENCES

[1] <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7674206/>, 2022/03/21

[2] https://www.epa.gov/sites/production/files/2015-10/documents/method_610_1984.pdf, 2022/03/21

