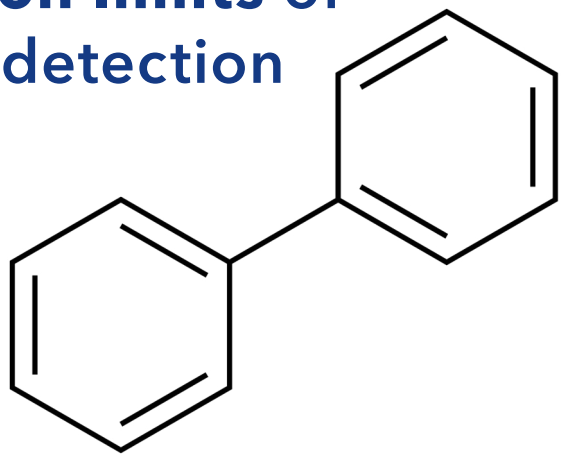
Detection and qualification limits of biphenyl with diode array detection



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SUMMARY

The use of the fungicide and food additive biphenyl has been severely restricted by the European Food Safety Authority (EFSA) since 2005. The multifunctional toxin is nevertheless found on numerous foodstuffs, which must therefore be strictly controlled. The RP HPLC method with simple UV detection shown here is a very fast and sensitive alternative to the previously used gas chromatography as a detection method.

INTRODUCTION

Biphenyl was particularly important in the period after World War II, as it enabled citrus fruits to be stored and transported over long distances. This meant that the valuable fruit could be imported to Europe from other continents and supply the suffering population with vitamins, especially in winter. As a fungicide, biphenyl effectively counteracts the formation of blue and green mold. Until its ban in 2005, biphenyl was labeled in the function as a preservative with the number E 202 and as the food additive E 230 [1]. Due to its effect, biphenyl is still used today as a component of pesticides. However, its use is prohibited in the EU. Accordingly, the limit value of biphenyl for spices and other foodstuffs was set at 0.01 and later at 0.05 mg/kg. Besides citrus fruits especially spices, teas and nutmeg are affected by the exclusion according to 91/414/EEC. Since in nutmeg the initially lower limit value could only be observed in exceptional cases, the value was corrected upwards by the European Food Safety Authority (EFSA) in 2010 [2]. Since biphenyl is also a versatile starting material for the pharmaceutical industry and is used, for example, in the production of liquid crystals in digital watches or pocket calculators, it can be detected as an environmental toxin. Biphenyl has been observed to cause eye and skin irritation and toxic effects on the liver, kidneys, and central and peripheral nervous systems. Kidney effects have been observed in long-term exposed animals [1].

Additional Information

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SAMPLE PREPARATION

Four stock solutions with different concentrations were prepared. For this purpose, the corresponding amount of solid was mixed with 100 ml methanol : water 50:50 (v/v) and placed in a 30 °C ultrasonic bath for 8 hours. Before analysis, the corresponding amounts were filtered through a hydrophilic nylon syringe filter with 0.2 µm pore size. The solutions should be stored at room temperature and for no longer than 24 hours.

RESULTS

For a calibration curve each concentration was weighed and dissolved separately, since precipitation was always observed during the dilution experiments. Three data points were recorded for each concentration. In each case, 10 µl were injected three times from one vial. Three levels were considered for the concentration curve, ranging from 0.01–10 µg/ml **(Fig. 1)**. The LOD and LOQ values were calculated and the experimental LOQ is confirmed in the chromatogram in **Fig. 2**.

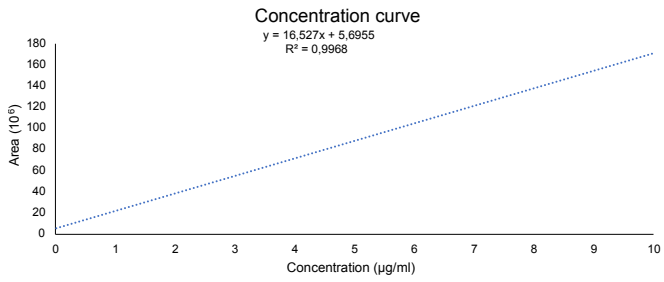

Fig. 1 Calibration curve of biphenyl in the range 0.01–10 µg/ml with 10 µl injection volume.

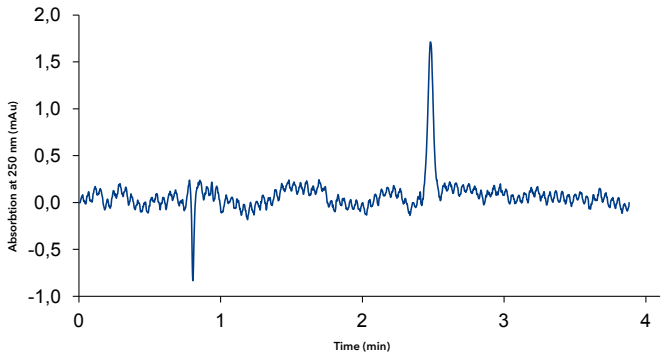

Fig. 2 Chromatogram of biphenyl with a 10 µl injection of a standard solution of 0.01 µg/ml.

Tab. 1 Calculated and empirical detection limits of biphenyl

Value	Concentration in µg/ml	S/N
LOQ (calculated)	0,0096	10
LOQ (empirical)	0,01	10,5
LOD (calculated)	0,0029	3

CONCLUSION

The fast 4 min RP method can be used to detect biphenyl very sensitively using UV detection. The limit of qualification (LOQ) is 0.01 µg/ml is by far sufficient to detect food contamination with the toxin. Furthermore, the more cost-effective and uncomplicated UV detection offers many advantages over the gas chromatography otherwise used for biphenyl.

MATERIAL AND METHODS

System configuration

Instrument	Description	Article No.
Eluent delivery	P 6.1L pump HPG with 10 ml pump head (stainless steel), 862 bar, degasser and mixer (100 µl)	APH35EA
Eluent delivery	Set of 4 bottles 1000 ml for solvent and 1 bottle 250 ml for back piston flushing GL45	A5324-2
Eluent delivery	Bottle tray E 2.1L	AZC00
Sample introduction	Autosampler up to 862 bar, rack 108 positions	AAA50AA
Columns and periphery	Column thermostat 5–85°C	ATC00
Columns and periphery	Eurospher II 100-5 C18, 250 x 4.6 mm	25EE181E2J
Detection	DAD 2.1L, Diode Array Detector DAD 2.1L without measuring cell, with test cell 190–700 nm	ADC01
Detection	50 mm, 6 µl DAD flow cell	AMD59XA
PC hardware and software	ClarityChrom 8.5 Station license for one system	A1670
PC hardware and software	ClarityChrom 8.5 PDA license for DAD data processing	A1676

Method parameters			
Eluent A	Water		
Eluent B	ACN		
Flow rate	2 ml/min		
Temperature	40° C		
Gradient	Time (min)	% A	% B
	0	20	80
	4	20	80
Injection volume	10 µl		
Detection	250 nm		
Data rate	10 Hz		
Time constant	0.1 s		

REFERENCES

[1] National Center for Biotechnology Information. "PubChem Compound Summary for CID 7095, Biphenyl" PubChem, https://pubchem.ncbi.nlm.nih.gov/compound/Biphenyl. Accessed 14 December, 2021.

[2] EFSA Journal Modification of the existing MRLs for biphenyl in various commodities 2010;8(10):1855.