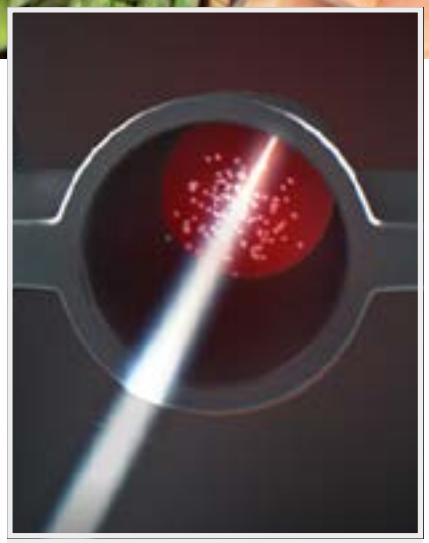

Determination of Toxic Traces of Cadmium, Lead, and Thallium in Food

Challenge

Determination of the toxic elements Cd, Pb, and Tl in food like grain, rice, vegetables, fish, and animal products; related to national and international standards.

Solution

Graphite furnance AAS with reliable Zeeman (ZEEnit) or spectral (contrAA 800) background correction for difficult matrices.

Introduction

The elements cadmium (Cd), lead (Pb), and thallium (Tl) are typical representatives of toxic elements. Above all these elements have chronic toxic effects on the human organism, in addition to acute toxic effects. Cadmium, for example, has mutagenic and reproductive effects, carcinogenic effects and influences the bone density. Lead, among others, has a particularly neurotoxic effect. The toxicity and the acute toxic effect of thallium is even higher than cadmium and lead. Thallium compounds are relatively volatile and are released in thermal processes such as incinerators, smelters, and cement factories. The thallium concentration in air, water, and soil increase and therefor thallium can accumulate in the food produced in such environment.

The intake of these toxic elements happens mainly through food, but also through the air e.g., cadmium in cigarette smoke. Since they can accumulate in the human organism it is hugely important to monitor traces of these elements in agriculturally grown foods such as cereals, rice, vegetables; but also in animal food such as fish, liver and other organ meats and their products.
Due to the sample matrix, the analysis by graphite furnace AAS can only be performed with appropriate background correction, such as the Zeeman background correction of the instrument ZEEnit 650P/700P or by means of spectral correction using the high-resolution continuum source graphite furnace AAS (HR-CS AAS) contrAA 800.

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Materials and Methods

Samples and reagents

Different certified reference materials (CRM) of fish and rice were analyzed for their content of Pb, Cd, and Tl by graphite furnace AAS using the ZEEnit and contrAA 800-series.
After microwave digestion the solutions were measured either directly or after further dilution. To monitor possible signal suppressing effects, caused by matrix components, the solutions were spiked with a defined analyte concentration (QC spike recovery). As there was no certified value available for Tl, the sample solutions were spiked with 5µg/L Tl.
The preparation of the calibration standards was performed by autosampler dilution of a pre-mixed stock solution.

Reagents

- HNO_3 65% suprapure (Merck)
- HCl 37% p.A. (Sigma Aldrich)
- H_2O_2 30% (Sigma Aldrich)
- $Pd(NO_3)_2$ matrix modifier (10 g/L, Sigma Aldrich)
- $Mg(NO_3)_2$ matrix modifier (10 g/L, Sigma Aldrich)
- Ascorbic acid (Merck)
- $NH_4H_2PO_4$ (Merck)
- Single element standard solutions for Pb, Cd, and Tl (1000 mg/L each, Merck)

Sample Preparation

Reference materials (CRM) of fish protein (DORM-4), dogfish liver (DOLT-5) and rice flour (IRMM-804) as well as commercially available rice grains were analyzed. The grains were homogenized prior to digestion by a ball mill.

The sample material was digested by using the microwave system TOPwave (vessel type PM60). Therefore, 7 mL HNO_3, 1 ml HCl and 2 mL H_2O_2 were added to approx. 0.6 g of each sample. After that it was heated in two steps at 170 °C for 15 min and at 200 °C for 20 min. Subsequently the digested solution was transferred into a volumetric flask and filled to 50 mL with deionized water. These solutions were either used directly for measurement or diluted with 0.5% HNO_3 suprapure.

Calibration

The calibration standards were prepared by autosampler dilution of a pre-mixed stock solution in 0.5% HNO_3 suprapure.
Table 1 shows the concentrations of the calibration standards.
For the determination of Cd in the fish samples (DORM-4, DOLT-5) the method of additions was used as well, since an aqueous standard calibration resulted in lower recoveries. Table 2 shows the preparation of the applied standard addition standards.

Table 1: Concentration of the calibration standards

Standard solution	Analyte concentration [µg/ L]		
	Pb	Cd	Tl
Stock solution	10	2	20
Cal. std. 0	0	0	0
Cal. std. 1	2	0.4	4
Cal. std. 2	4	0.8	8
Cal. std. 3	6	1.2	12
Cal. std. 4	8	1.6	16
Cal. std. 5	10	2	20

Table 2: Calibration standards for standard addition procedure (Cd in fish), injection volume: 15 µL sample + 15 µL standard

Standard solution	Analyte concentration [µg/ L]
	Cd
Stock solution	2
Sample	0
+ Add1	0.8
+ Add2	1.6

Modifier

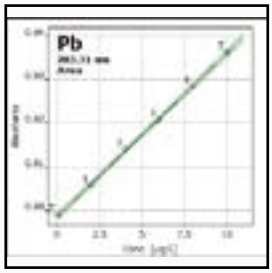
Table 3 shows the used modifiers, which are added automatically by the autosampler from a stock solution.

Table 3: Modifier for ZEEnit and contrAA 800:

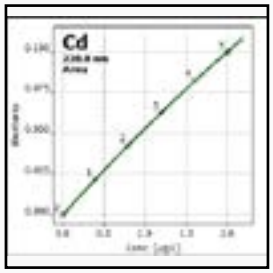
Element	Modifier
Pb	5 µL $NH_4H_2PO_4$ 1%
Cd	5 µL $NH_4H_2PO_4$ 1%
Tl	5 µL $Pd/Mg(NO_3)_2$ 0.1/0.05%, 5 µL ascorbic acid 1% (injection after sample)

Note: By using a palladium modifier for analysis of Cd in rice, the measurement results were found to be significantly lower (recovery rate around 50 to 70%). For Cd in fish this modifier type can be used alternatively.

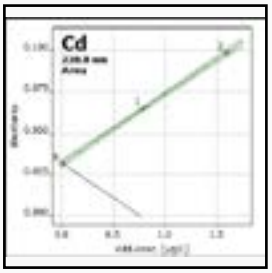
Resulting calibration functions with ZEEnit are shown in Figure 1 and with contrAA are shown in Figure 2.



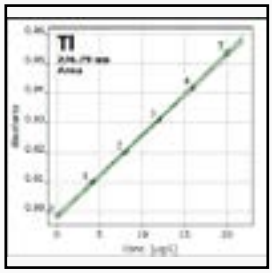
linear; R^2 (adj.)=0.9988
standard calibration



nonlinear; R^2 (adj.)=0.9998
standard calibration

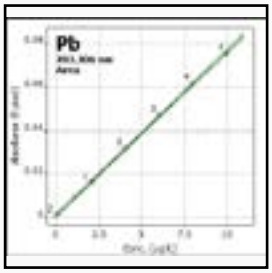


linear; R^2 (adj.)=0.9999
resulting calibration functions
(methods of additions) Dolt 5

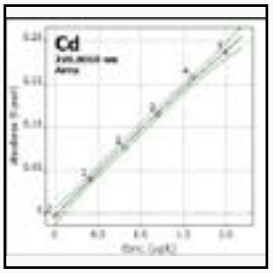


linear; R^2 (adj.)=0.9991
standard calibration

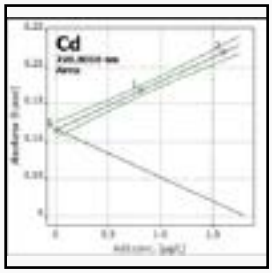
Figure 1: Resulting calibration functions with ZEEnit



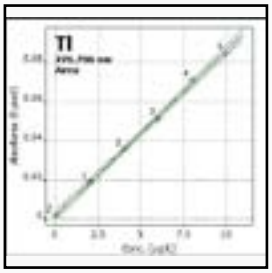
linear; R^2 (adj.)=0.9993
standard calibration



nonlinear; R^2 (adj.)=0.9972
standard calibration



linear; R^2 (adj.)=0.9997
resulting calibration functions
(methods of additions) Dorm 4



nonlinear; R^2 (adj.)=0.9982
standard calibration

Figure 2: Resulting calibration functions (standard calibration) with contrAA 800

Instrumentation

The following tables list the used accessories and method settings.

Table 4: General instrument parameters and accessories

Parameter	Specification ZEEnit	Specification contrAA 800
Instrument	ZEEnit 650 P	contrAA 800 G
Autosampler	AS-GF	AS-GF
Tube type	PIN platform, transversely heated	PIN platform, transversely heated

Table 5: Instrument and evaluation parameters for ZEEnit

Element	Wavelength [nm]	Slit [nm]	Lamp current [mA]	Background correction	Magnetic field strength [T]	$T_{Pyr.}$ [°C]	$T_{Atomis.}$ [°C]	Meas.time [s]
Pb	283.3	0.5	2.0	Zeeman, 2-field mode	0.8	800	1600	4.0
Cd	228.8	0.5	2.0	Zeeman, 2-field mode	0.8	600	1600	4.0
Tl	276.8	0.5	4.0	Zeeman, 2-field mode	1.0	550	1950	4.0

Table 6: Instrument and evaluation parameters for contrAA 800

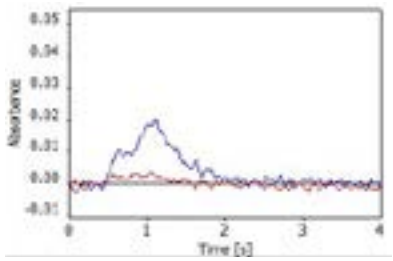
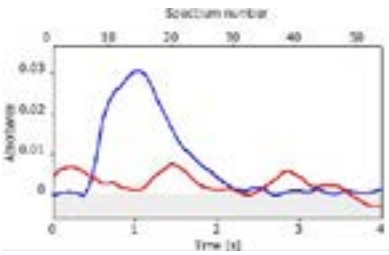
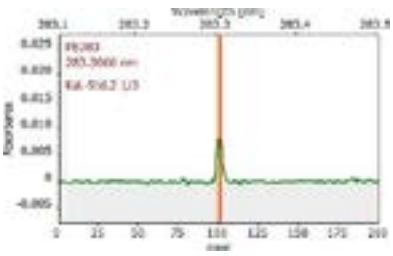
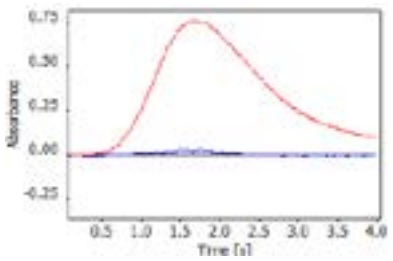
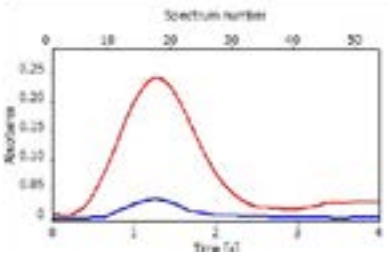
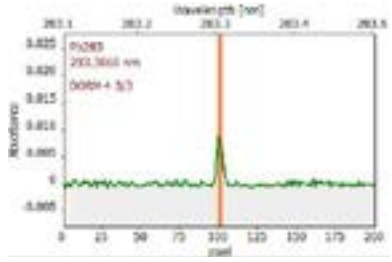
Element	Wavelength [nm]	No. of eval. pixels	$T_{Pyr.}$ [°C]	$T_{Atomis.}$ [°C]	Ramp [°C/s]	Meas.time [s]	Baseline correction
Pb	283.3060	5	800	1700	1500	5.0	IBC, LSBC
Cd	228.8018	5	600	1600	1400	3.0	IBC, LSBC
Tl	276.7860	5	550	1950	1500	4.0	IBC, LSBC

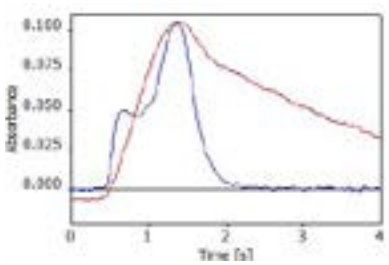
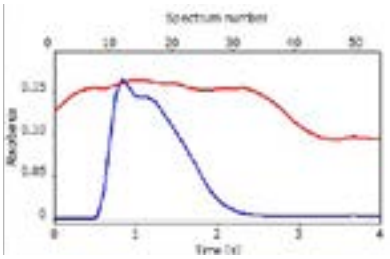
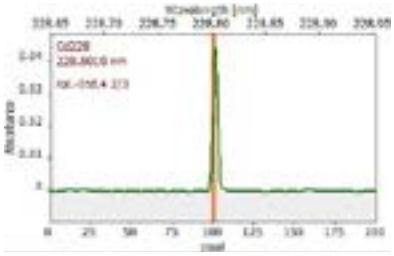
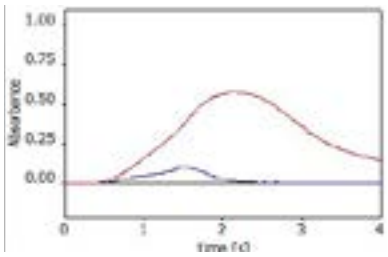
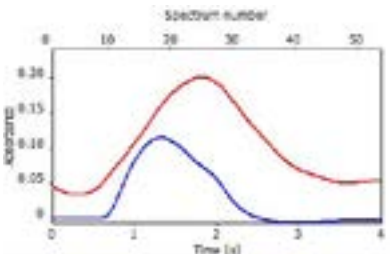
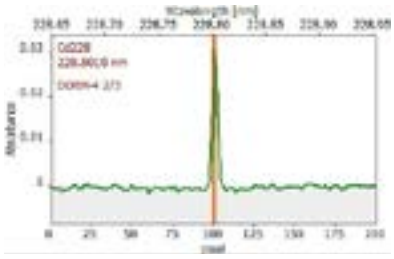
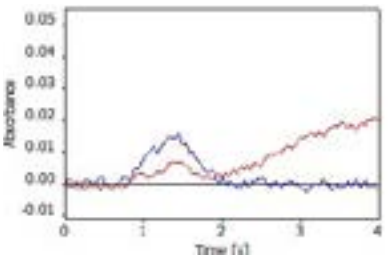
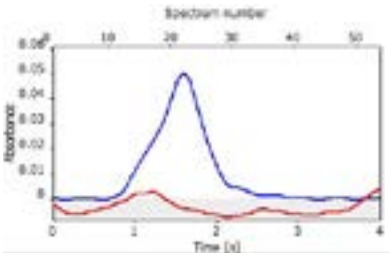
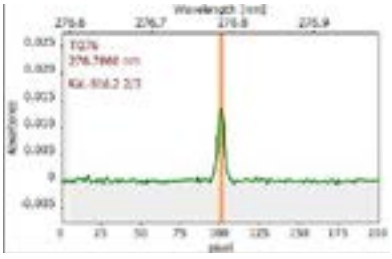
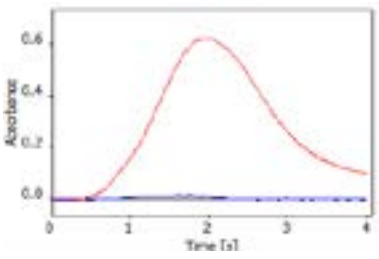
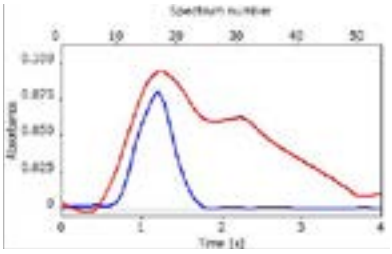
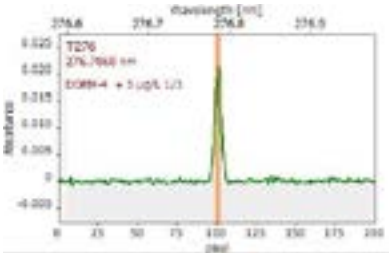
IBC: Iterative Base Line Correction
LSBC: Least Squares Background Correction

Results and Discussion

Typical signals with the ZEEnit and the contrAA 800

Table 7: Characteristic signal shapes and the contrAA 800 specific spectral vicinity of the applied analyte line

Element	ZEEnit measurement signal	contrAA 800 measurement signal	contrAA 800 2D spectrum
Pb Cal.std. 4 µl/L			
Sample DORM - 4			

Element	ZEEnit measurement signal	contrAA 800 measurement signal	contrAA 800 2D spectrum
Cd			
Cal.std. 1.6 µl/L			
Sample DORM - 4			
Tl			
Cal.std. 4 µl/L			
Sample DORM - 4 + 5µg/L Tl			

blue: analyte signal
red: background signal

Note: Undiluted or only slightly diluted sample solutions can show a spectral interference with direct analyte peak overlapping of diatomic molecule bands, caused by sample matrix components. This can lead to false high results, in particular at very low analyte concentrations. Hence, the use of "Least Squares Background Correction" (LSBC) is recommended.

Measurement results

Table 8 shows the measurement results for the samples. In order to check for a possible matrix influence on the signal intensity, each sample was spiked with a defined analyte concentration. The measured QC spike recovery rates are included in the following table, as well as the recovery of the certified values of the CRM. For Cd the best CRM recoveries for the fish samples (DORM-4, DOLT-5) were obtained by applying the method of additions. Thus, the results for Cd in fish are given for this calibration procedure. In the case of Tl no certified concentrations were available. The concentration of Tl in all samples was below the limit of detection. Hence, each sample solution was spiked with 5 µg/L Tl.

Table 8: Measuring results and QC spike recovery

Sample				ZEEnit			contrAA 800		
Name	**Element**	**DF**	**Certified value [mg/kg]**	**Measured analyte concentration**	**RSD [%]**	**Recovery of CRM value/ QC spike [%]**	**Measured analyte concentration**	**RSD [%]**	**Recovery of CRM value/ QC spike [%]**
DORM-4 (CRM, Fish protein)	Pb	1	0.404	0.433 mg/kg	1.8	CRM: 107	0.436 mg/kg	1.9	CRM: 108
	Cd	2	0.299	0.309 mg/kg	0.3	CRM: 103	0.304 mg/kg	1.4	CRM: 102
+ 5 µg/L Tl	Tl	1	–	4.84 µg/L	0.5	QC: 96.8	4.80 µg/L	2.8	QC: 96.0
DOLT-5 (CRM, Dogfish liver)	Pb	1	0.162	0.162 mg/kg	3.5	CRM: 100	0.159 mg/kg	1.2	CRM: 98.1
	Cd	200	14.5	13.7 mg/kg	0.7	CRM: 94.5	14.4 mg/kg	0.8	CRM: 99.3
DOLT-5 + 5 µg/L Tl	Tl	1	–	4.76 µg/L	0.8	QC: 95.2	5.15 µg/L	1.8	QC: 103
IRMM-804 (CRM, Rice flour)	Pb	1	0.42	0.400 mg/kg	0.6	CRM: 95.2	0.398 mg/kg	0.8	CRM: 94.8
	Cd	20	1.61	1.62 mg/kg	0.2	CRM: 101	1.53 mg/kg	0.9	CRM: 95.0
IRMM-804 + 5 µg/L Tl	Tl	1	–	4.88 µg/L	3.2	QC: 97.6	4.79 µg/L	1.4	QC: 95.8
Rice grains	Pb	1	–	< LOD	–		< LOQ	-	
	Cd	1	–	0.019 mg/kg	3.5		0.021 mg/kg	3.6	
Rice grains+5 µg/L Tl	Tl	1	–	4.77 µg/L	2.3	QC: 95.4	4.80 µg/L	2.5	QC: 96.0

DF: manual dilution factor
LOD: Limit of detection
LOQ: Limit of quantification

Simultaneous multi-line evaluation with the contrAA 800

Compared to conventional AAS systems, the High-Resolution Continuum Source AAS provides further opportunities for analysis. For example, different analyte lines, which appear in the same observation range of 200 pixels, can be combined and evaluated simultaneously under specific conditions. For this purpose, one analyte line is defined as a main measurement line in the method, the values for the second line are subsequently reprocessed automatically.

This feature can be demonstrated for determination of low concentrations of Cd (primary line at 228.8018 nm) and high concentrations of Fe (insensitive secondary line at 228.7250 nm) in DORM-4. The following method settings were applied for this purpose (Table 9).

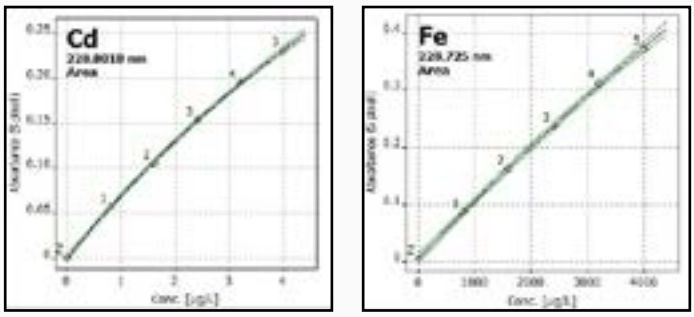
Table 9: Instrument and evaluation parameters for simultaneous evaluation

Element	Wavelength [nm]	No. of eval. pixels	$T_{Pyr.}$ [°C]	$T_{Atomis.}$ [°C]	Ramp [°C/s]	Meas.time [s]	Modifier	Baseline correction
Cd (main line)	228.8018	5	600	1650	1400	9	5 µL Pd/ $Mg(NO_3)_2$ 0.1/0.05 %	IBC, LSBC
Fe (additional line)	228.7250							

For calibration a stock standard, containing 4 µg/L Cd and 4000 µg/L Fe was diluted by the autosampler. The resulting concentrations (Table 10) and calibration curves (Figure 5) are shown below.

Table 10: Concentration of the calibration standards for simultaneous evaluation

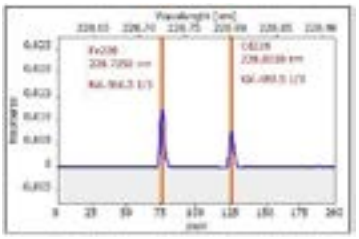
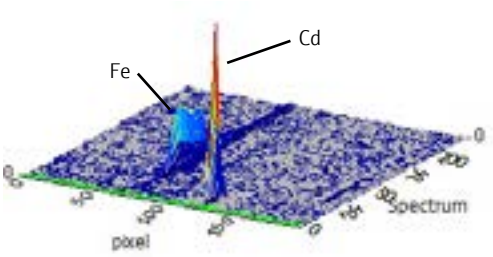
Standard solution	Analyte concentration [µg/ L]	
	Cd	Fe
Stock solution	4	4000
Cal. std. 0	0	0
Cal. std. 1	0.8	800
Cal. std. 2	1.6	1600
Cal. std. 3	2.4	2400
Cal. std. 4	3.6	3600
Cal. std. 5	4	4000



nonlinear; R^2(adj.)=0.9997 nonlinear; R^2(adj.)=0.9993

Figure 5: Resulting calibration functions (standard calibration)

Table 11: Characteristic signal shapes and spectral vicinity of the applied analyte line for simultaneous evaluation with contrAA 800

Element	2D spectrum	3D spectrum
Fe **Cd**		

Conclusion

The obtained analyte recoveries by using the described methodology demonstrates the suitability of the ZEEnit and contrAA800 series for this application.

Recovery rates of the CRM value were similar for both systems, summarized in Table 12. Hereby, it was found that a measurement of Cd in the digested fish samples (DORM-4, DOLT-5) gave lower CRM value recoveries (QC spike recovery of approx. 80%). For this reason, the method of additions was applied for calibration for this measurement task.

Table 12: Summary of the recovery rates

Recovery rates	Cd	Pb	Tl (QC Spike)
Fish CRM	95 – 103%	98 – 108%	95 – 103%
Rice CRM	95 – 101%	95%	96 – 98%

Both the ZEEnit series with Zeeman background correction and contrAA 800 series with spectral background correction are excellent for determining the toxic trace elements Cd, Pb, and Tl in food. Hereby, the limits of detection and quantification of the contrAA 800 are even lower than these of the ZEEnit.

The contrAA800 with its Xe short-arc lamp as a continuum light source in the HR-CS AAS offers an additional advantage. It enables the simultaneous measurement of additional elements like iron using the simultaneous multi-line evaluation.

Table 16: Advantages and detection limits by device

	novAA 800	ZEEnit series	contrAA 800
			
Advantages	■ Not recommended due to spectral interferences	■ Reliable Zeeman background correction with magnetic field up to 1 Tesla ■ Autosampler AS-GF for automatic calibration and sample dilution ■ ONE + ONE atomizer compartment for more applicable flexibility ■ Possibility for direct solid sampling	■ HR-CS AAS with simultaneous spectral background correction ■ Autosampler AS-GF for automatic calibration and sample dilution ■ Dual atomizer conzept allows also flame application ■ Possibility for direct solid sampling ■ Best detection limits ■ Simultaneous multi-line evaluation for multi element measurement
Detection limits			
Cd	-	0.009 µg/L	0.01 µg/L
Pb	-	0.33 µg/L	0.03 µg/L
Tl	-	0.65 µg/L	0.04 µg/L

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