Post-column chemistry for improved optical absorption detection

N. Seifert, J. Kleimann, H. Krienbühl, and K.M. Ruth

Ion Chromatography

Summary

UV/VIS detection is one of the most sensitive detection techniques in trace-level chromatography. Sometimes, however, spectrophotometric detection lacks sensitivity, selectivity or reproducibility and chemical derivatizations are required. By using Metrohm`s rugged and versatile flow-through reactor, single- or multi-step derivatizations can be done fully automatically, in either pre- or post-column mode at any temperature between 25…120 °C. The variable reactor geometry allows to adjust the reactor residence time of the reactants according to derivatization kinetics. The flexibility of the reactor is demonstrated by optimizing four widespread post-column techniques: the relatively slow ninhydrin reaction with amino acids and the fast derivatizations of silicate, bromate, and chromate(VI).

Introduction

When analytes show weak or no absorption in the UV/VIS region of the electromagnetic spectrum, derivatization and complexation reactions are used to generate chromophores for enhanced spectrophotometric detection. Since chromatographically separated analytes are derivatized before entering the detector, signal strength is improved without compromising chromatographic separation.

For inorganic ions, the typical photometry reagents such as 4-(2'-pyridylazo)-resorcinol (PAR) among many other complexing agents can be used. For organic compounds, a great choice of suitable derivatization reagents are available. For method development, the choice of reagent as well as the reaction parameters are most important and subject to optimization. When the reagent is not selective, it can be used for many compounds of similar structure. A typical example is PAR that forms complexes with many metal ions and allows trace analysis of the whole group of transition metals due to improved optical absorption of the complexes formed. In this case, the separation step is crucial and often the limiting factor. When the reagent is very selective, less performance is required in the separation step. Since this type of reagent does not react with matrix or excess components, traces can be selectively detected directly within the matrix. In the case of selectivity for only one analyte, no separation is necessary and pure optical methods come into play. Typical examples of very high selectivity are molybdophosphoric acid-based applications.

In our presentation we show improved equipment for post-column chemistry suited for any liquid chromatography system. Methods were developed and optimized in terms of reagent concentration, flow rates, and reactor residence time of the resulting mixture. Results are shown for the trace-level determination of amino acids, silicate, chromate(VI), and bromate.

Instrumentation

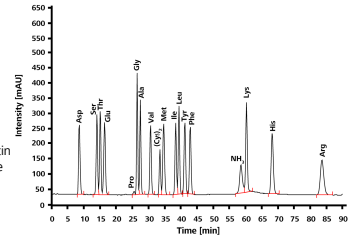
- 850 Professional IC
- 889 IC Sample Center
- 872 Extension Module IC Pump
- 886 Professional Reactor
- 887 Professional UV/VIS Detector

Amino acids (AA) according to USP

The determination of the amino acid composition, whether in hydrolyzed proteins and peptides or in USP-regulated pharmaceuticals, is crucial. One of the most accurate chromatographic methods involves post-column ninhydrin derivatization.

0.25 µmol/L AA standard

Column:	Metrosep Amino Acids 1 - 100/4.0
Column temp.:	50 °C
Eluent A:	1.4% lithium citrate (pH = 2.8, HCl)
Eluent B: (gradient)	1.4% lithium citrate + 4.3% lithium chloride (pH = 4.5, HCl)
Flow:	0.4 mL/min
Loop:	20 µL
PCR volume:	0.39 cm³, 0.5 mm i.d. × 2 m
PCR reagent:	4.0 g ninhydrin and 0.16 g hydrindantin in 154 g DMSO, 1.4 g lithium acetate dihydrate, 0.2 g acetic acid (37%), and 45 g ultrapure water
PCR flow:	0.2 mL/min
PCR temp.:	120 °C
Wavelength:	570 nm

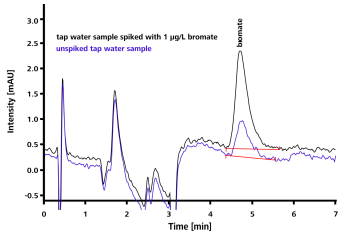


Bromate according to EPA 326.0

Ozonolysis of bromide-containing drinking waters results in the formation of the potential carcinogen bromate. It can be effectively detected at trace-levels by post-column derivatization with iodide as described in the U.S. EPA Method 326.

Spiked (1 µg/L) and unspiked tap water sample

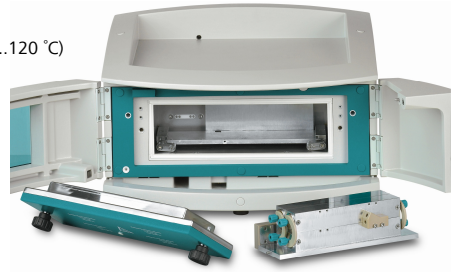
Column:	Metrosep A Supp 16 - 100/4.0
Column temp.:	45 °C
Eluent:	100 mmol/L sulfuric acid 19.3 µmol/L ammonium hepta-molybdate tetrahydrate
Flow:	0.8 mL/min
Loop:	10 µL
PCR volume:	0.39 cm³, 0.5 mm i.d. × 2 m
PCR reagent:	0.27 mol/L potassium iodide
PCR flow:	0.2 mL/min
PCR temp.:	25 °C
Wavelength:	352 nm



886 Professional Reactor

Specifications

- heatable pre-column and post-column reactor (25...120 °C)
- customizable reactor geometry
- flow-through reactor with minimized dead volume
- robust, highly pH- and solvent-resistant reactor
- metall-free flowpath
- intelligent reactor (iReactor)



Benefits

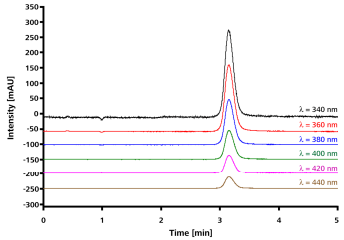
- broad application range
- high derivatization yields, excellent detection limits
- outstanding separation performance
- ideally suited for complex matrices
- derivatizations at high temperatures
- guarantees traceability, reduces operating errors

Silicate

In the semiconductor and power industry the use of ultrapure water is imperative. Silica impurities are ubiquitous and can be effectively determined by ion chromatography followed by UV/VIS detection after post-column reaction with molybdate.

50 mg/L silicate standard

Column:	Hamilton PRP-X100 - 125/4.0
Column temp.:	30 °C
Eluent:	4.0 mmol/L sodium hydroxide 0.4 mmol/L sodium carbonate
Flow:	1.5 mL/min
Loop:	10 µL
PCR volume:	0.78 cm³, 0.5 mm i.d. × 4 m
PCR reagent:	20 mmol/L sodium molybdate dihydrate and 0.2 mol/L HNO_3
PCR flow:	0.5 mL/min
PCR temp.:	30 °C
Wavelength:	340…440 nm (best sensitivity at 360 nm)

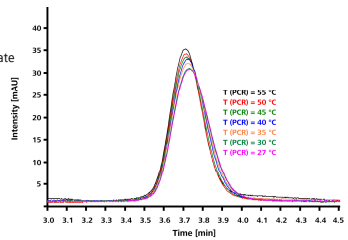


Chromate(VI) according to EPA 218.6

Hexavalent chromate is highly toxic and carcinogenic. Chromatographic determination implies post-column reaction (PCR) with diphenylcarbazide according to U.S. EPA Method 218.

1 mg/L chromium standard according to EPA 218.6

Column:	Metrosep A Supp 5 - 100/4.0
Column temp.:	25 °C
Eluent:	12.8 mmol/L sodium carbonate 4.0 mmol/L sodium hydrogen carbonate
Flow:	0.8 mL/min
Loop:	10 µL
PCR volume:	0.39 cm³, 0.5 mm i.d. × 2 m
PCR reagent:	0.5% diphenylcarbazide 0.5 mol/L sulfuric acid 10% methanol
PCR flow:	0.5 mL/min
PCR temp.:	27…55 °C (best sensitivity at 55 °C)
Wavelength:	540 nm



Metrohm International Headquarters, CH-9101 Herisau/Switzerland, nsf@metrohm.com