Analytical method development and validation of on-line sample processing methods

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Contents

On-line measurements: What do we need?

⇒ **Automated methods: Minimum operational maintenance. Implementation of analytical process in a single protocol**

⇒ **Miniaturization: Decreased consumption of chemicals and samples**

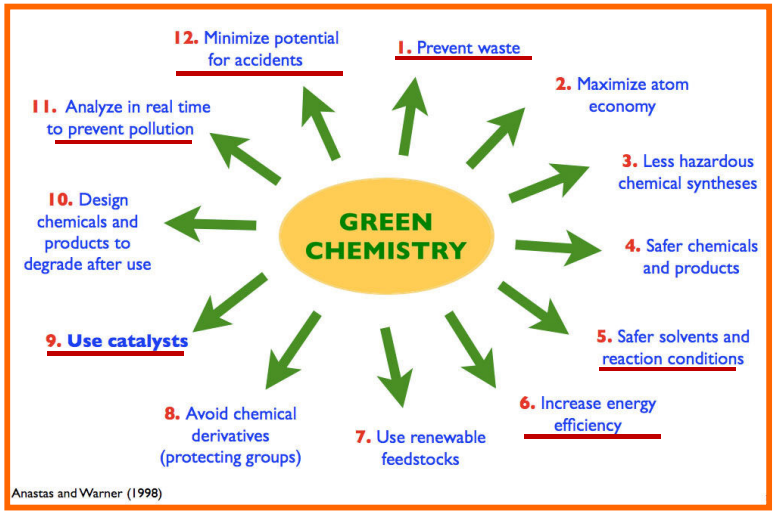
⇒ **Throughput: High sampling frequency for improved method efficiency**

⇒ **Portability: Monitoring of parameters (*in-situ*)**

⇒ **Sample processing: Clean-up, separation and preconcentration.**

⇒ **Implementation of QC/QA tools. Environmental management systems: ISO 14001. 12th principles Green Chemistry**

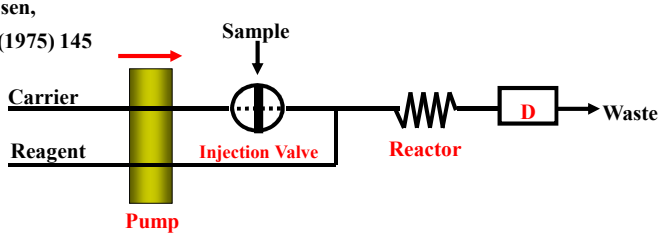
Principles of Green Chemistry- Role automatic and miniaturized methods



Automatic assays by flow analysis

1st generation of flow injection: Flow Injection Analysis

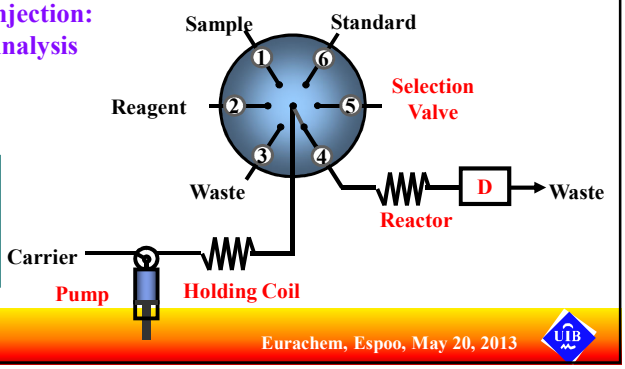
J. Ruzicka, E.H. Hansen,
Anal. Chim. Acta, 78 (1975) 145



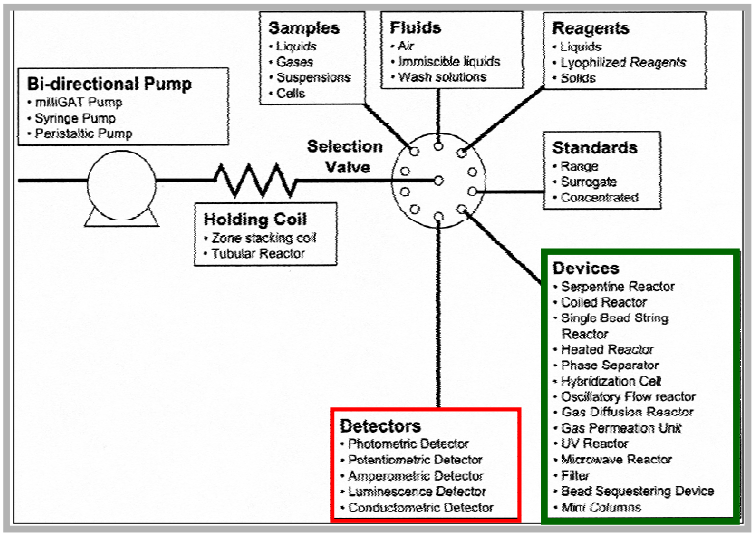
2nd generation of flow injection: Sequential Injection Analysis

J. Ruzicka, G.D. Marshall,
Anal. Chim. Acta, 237 (1990) 329

Programmable flow:
unidirectional flow
bi-directional flow
stopped flow

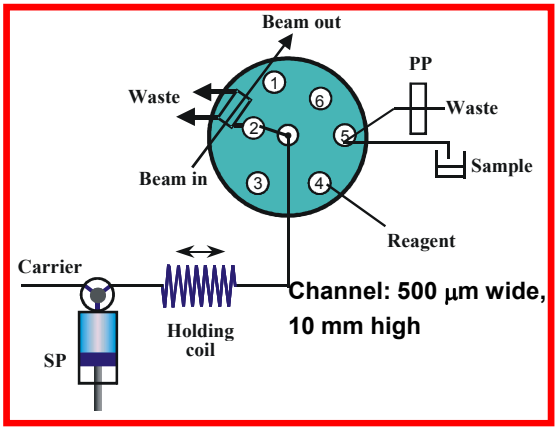


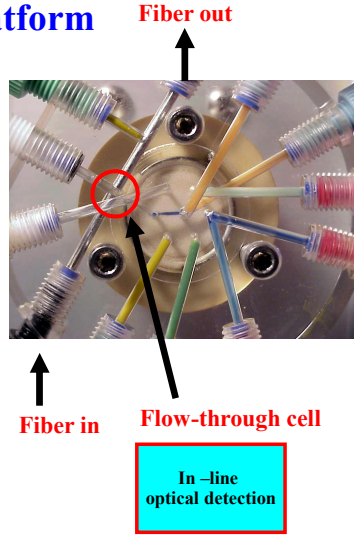
Versatility of Sequential Injection Analysis



G.D. Marshall et al., *Anal. Chim. Acta*, 499 (2003) 29

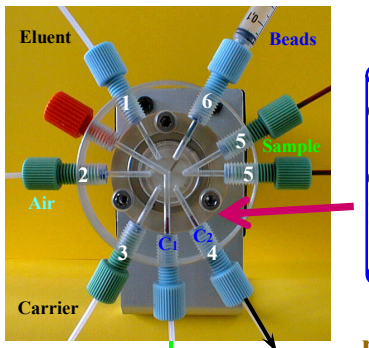
3rd Generation of flow injection: Lab-on-a-valve (LOV) microfluidic platform

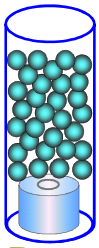




J. Ruzicka, Analyst 125 (2000) 1053

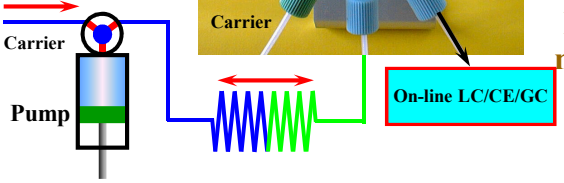
LOV - Bead Injection





Automatic single use µSPE (≤ 7 mg sorbent)

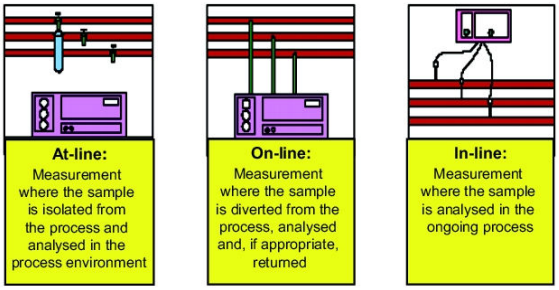
Renewable microcolumn



In-valve bead manipulation: Rod, frit or optical fiber restriction

M. Miró, E.H. Hansen, Anal. Chim. Acta, 750 (2012) 3-15

Coupling of flow systems with analytical instrumentation (3 levels of automation)



FLOW SYSTEMS :

at-line: automatic collection eluate/sample into an autosampler vial and robotic injection into the detector (no direct hyphenation)

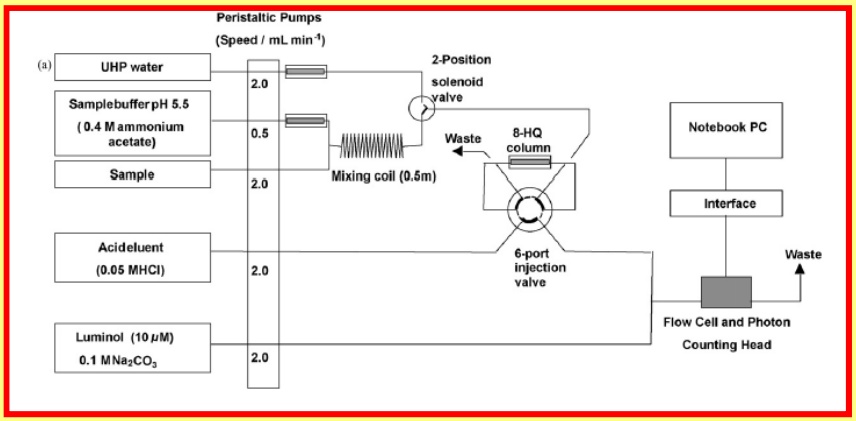
on-line: interfacing the flow system and detector via a rotary valve (FI-HPLC, FI-FAAS) or transfer line (FI-ETAAS)

in-line: integration of detector in the flow system

Standard methods for environmental parameters

Parameter	Official method	Detection	Method/reaction	Dynamic linear range (mg/l)	Water matrices
Ammonium	EN/ISO 11732 (1997)	Photometry	Gas-diffusion (color change of pH indicator)	0.1-1.0 1.0-10	Ground, drinking,surface and wastewater
Nitrite	EN/ISO 13395 (1996)	Photometry	Griess-Ilosvay	0.01-0.1 0.1-1.0	Drinking, surface,ground and wastewater
Oxidised nitrogen	EN/ISO 13395 (1996)	Photometry	Cu-Cd reduction column/ Griess-Ilosvay	0.2-20 2.0-20	Ground, drinking,surface and wastewater
Orthophosphate	EN/ISO 15681 (2001)	Photometry	Molybdenum blue	0.01-1.0	Drinking, surface, ground and wastewater
Dissolved organic phosphorous	EN/ISO 15681 (2001)	Photometry	UV-photooxidation/ Molybdenum blue	0.1-10	Drinking, surface, ground and wastewater
Chloride	EN/ISO 15682 (2000)	Potentiometry and photometry	Mercury(II)thiocyanate (photometry) Ion-selective electrode (potentiometry)	1.0-10 10-100 100-1000	Surface, ground, drinking, wastewater
Cyanide (total cyanide)	EN/ISO 14403 (2000)	Photometry and amperometry	Gas-diffusion/ Chloramine-T method (photometry) UV-irradiation for total cyanide	0.01-0.1	Industrial effluents, waste, ground and surface

On-line/in-situ seawater measurements



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On-line/in-situ seawater measurements

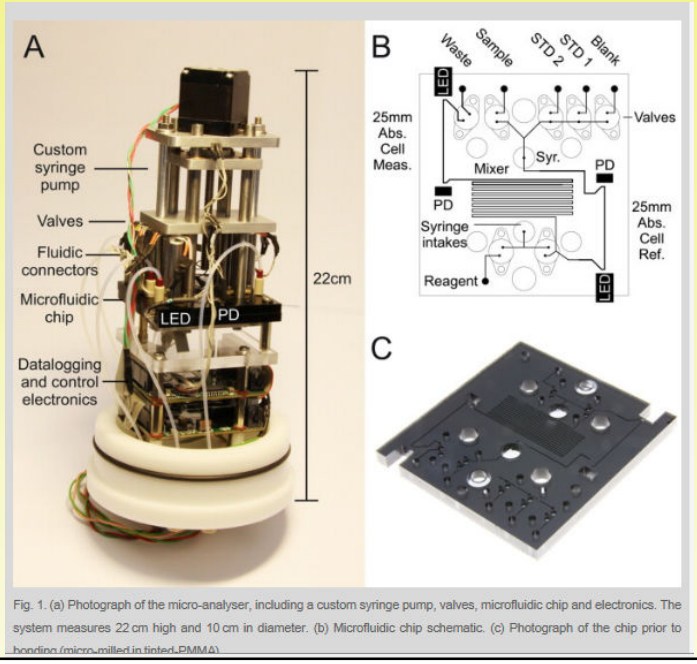
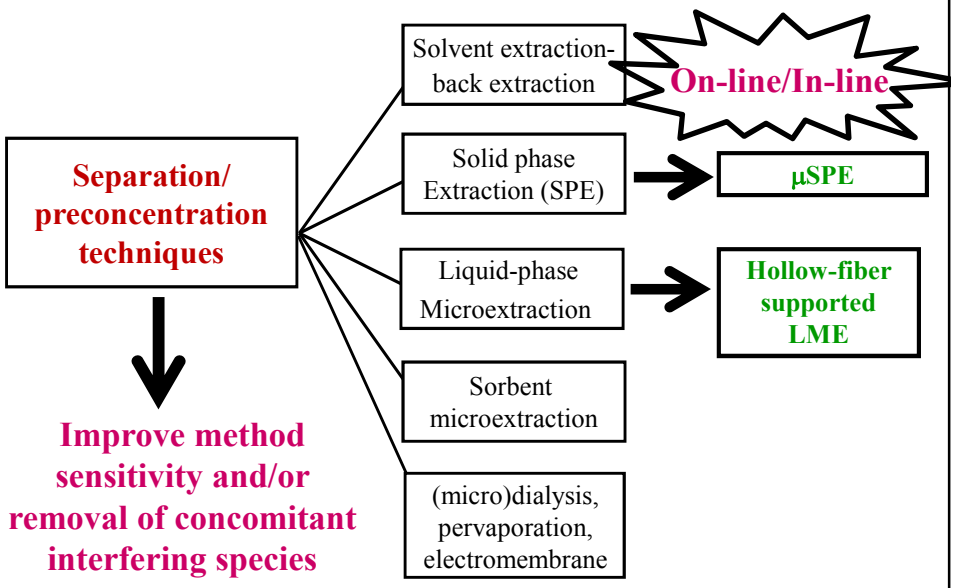


Fig. 1. (a) Photograph of the micro-analyser, including a custom syringe pump, valves, microfluidic chip and electronics. The system measures 22 cm high and 10 cm in diameter. (b) Microfluidic chip schematic. (c) Photograph of the chip prior to bonding (micro-milled in tinted PMMA).

Sample processing prior to analyte detection



Method validation (single lab)

Distinctive parameters in on-line sample preconcentration:
Enrichment factor (EF), Enhancement factor (N), Retention efficiency (Ref), Concentration efficiency (CE) and Consumptive index (CI)

1. $EF=C_e/C_s=$ Slope$_{ap}$/Slope$_{wp}$ (same experimental conditions)
2. $N_t=N_1 \cdot N_2 \cdot N_3 \cdot N_n \cdot EF$
3. $R_{ef}=m_e/m_s$ $\quad EF= V_s/V_e \times R_{ef}$
4. $CE=EF\ (f/60)$ $\quad$ f should include replicate injections and calibration time!!!
5. $CI=V_s/EF$

Analytical validation in on-line preconcentration procedures

Validation check-list: Fit for purpose!!!

1. Linear range (sensitivity): Lack of fit ($p > 0.05$) better than r^2 *(standards processed like samples!!!)*

2. LOD: S/N=3, $3s_{blank}$ criterion or $S_{y/x}$ criterion

3. Repeatability: Replicate injections using a single preconcentration column or single membrane separation unit

4. Intermediate precision: Measurement of a set of samples using different preconcentration columns or membranes

Analytical validation of on-line preconcentration procedures

Validation check list: Fit for purpose!!!

5. Selectivity: Concentration of interfering species tolerated at the 5 or 10 % interference level (doped samples or standards)- Compare improvement against direct sample injection

6. Ruggedness: Variation of signal (±5-10%) upon slight change of experimental parameters. Recommendation: Surface response models for method optimization

7. Trueness: CRM (same matrix!!!), reference standard method or doped samples (relative recoveries)

CASE study: µSPE for low abundance determination of Cr(III) and Cr(VI) in hardwaters

Fundamental principles:

✓ Selective retention of Cr(III) onto a miniaturized Chelating Sepharose microcolumn in LOV (against alkaline-earth metals).

✓In-line reduction of Cr(VI) to Cr(III) using aqueous hydroxylamine in open-tubular reactor

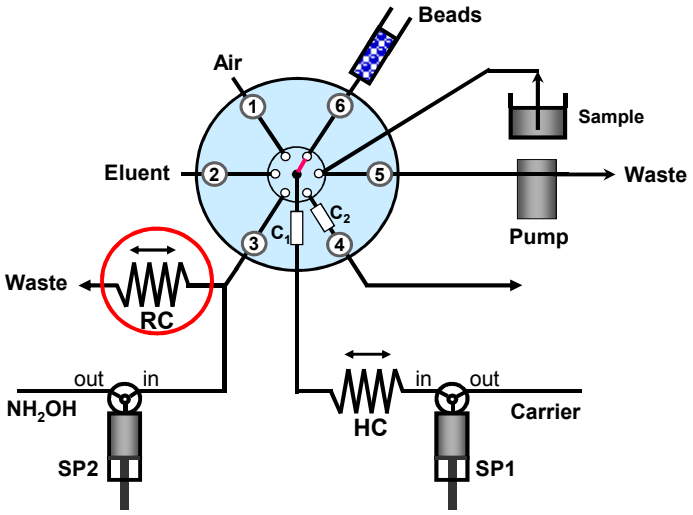
✓Sequential determination of Cr(III) and total Cr

✓On-line injection of eluate into ETAAS

✓ Aim: Preconcentration of Cr for determination below MAC (WHO: 50 ppb for Cr, EPA: 100 ppb for Cr, New Public Health Goal in California of 20 ppt Cr(VI))

X.-B. Long, M.Miró, E.H. Hansen, *J. Anal. At. Spectrom.*, 20 (2005) 1203

CASE study: µSPE for low abundance determination of Cr(III) and Cr(VI) in hardwaters



X.-B. Long, M. Miró, E.H. Hansen, *J. Anal. At. Spectrom.*, 20 (2005) 1203

CASE study: µSPE for low abundance determination of Cr(III) and Cr(VI) in hardwaters

Parameter	Cr(III)	Cr(VI)
Regression equation	1.0792 [Cr(III), µg L^{-1}]	0.7380 [Cr(VI), µg L^{-1}] – 0.0008
Correlation coefficient	0.9988	0.9990
Linear range/µg L^{-1}	0.02–0.28	0.035–0.4
Sample volume/mL	1.8	1.8
Loading flow rate/mL min^{-1}	4.5	4.5
Maxium injection throughput/h^{-1}	12	8
Eluent volume/µL	25	25
Retention efficiency (%)	86	—
Reduction efficiency (%)	—	68
Enrichment factor	62	42
Concentration efficiency/min^{-1}	12.4	5.6
Detection limit/µg L^{-1} (3σ)	0.010	0.020
Repeatability (%, 0.2 µg L^{-1}, $n = 7$)	2.4	2.2
Reproducibility (%, 0.2 µg L^{-1}, $n = 6$)	4.7	4.5

CRM: 1640-Trace elements in Natural Water ($t_{exp} < t_{crit}$)

X.-B. Long, M.Miró, E.H. Hansen, *J. Anal. At. Spectrom.*, 20 (2005) 1203

CASE study: In-line LPME for separation and determination of Cr(VI) in soil extracts

Fundamental principles:

✓ Clean-up of soil extracts (elimination of organic matter effects) for Cr(VI) analysis using Diphenylcarbazide (DPC) method.

✓Hollow-fiber supported liquid membrane extraction containing anion-exchange ionic liquid in kerosene to extract Cr(VI) and release in the acceptor containing DPC

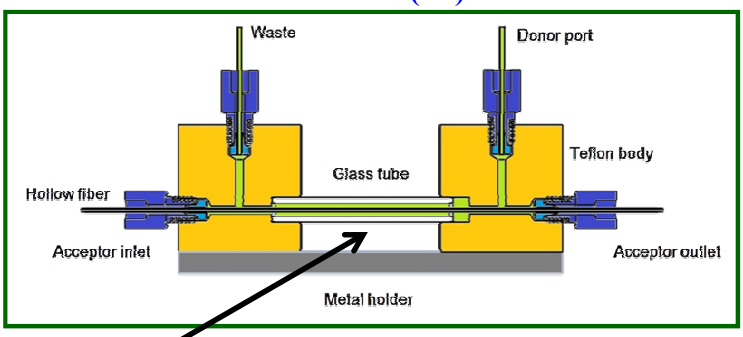
✓Design of a portable analyzer for potential in-field screening of Cr(VI) in soils

✓Automated membrane regeneration for unattended analysis

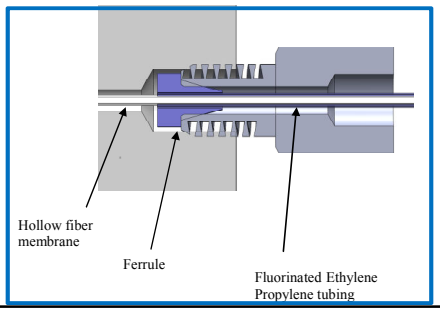
✓ Aim: Isolation of Cr(VI) from humic/fulvic acids affecting spectrophotometric detection

S. Nitiyanontakit, P. Varanusupakul, M. Miró, *Anal. Bioanal. Chem.*, 405 (2013) 3279

CASE study: In-line LPME for separation and determination of Cr(VI) in soil extracts



Hollow-fiber



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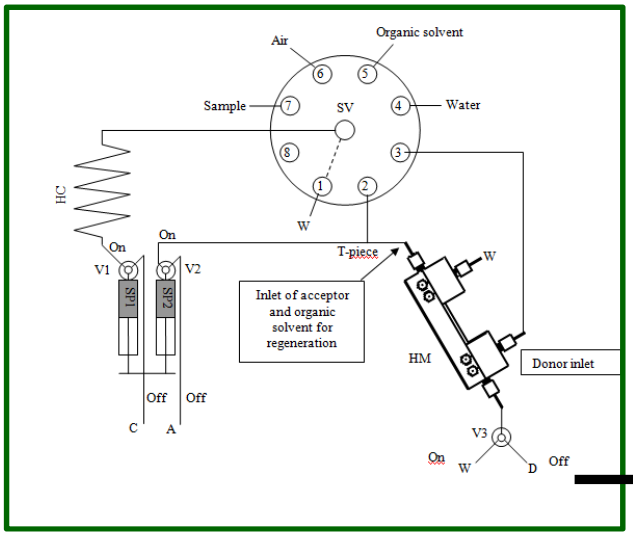

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Analytical method performance

Analytical parameter	Value
Calibration curve	y=0.8881x-0.0026; y= absorbance (AU); x= [Cr(VI)] in mg L^{-1} (R^2 = 0.9963)
Linear working range	30 – 500 µg L^{-1}
LOD	4.6 µg L^{-1}
LOQ	15.3 µg L^{-1}
Repeatability (250 µg L^{-1}, n=7)	4.2 %
Intermediate precision (250 µg L^{-1}, 5 days)	9.6 %
Extraction efficiency (%)	13.2
Enrichment factor	10.9

Extraction time and membrane regeneration: 14 min

Sample Volume: 2.8 mL

2710 (NIST soil) preceded by the EPA alkaline digestion method 3060A- Need for standard addition method

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Validation of on-line preconcentration methods

✓ Optimization of analytical properties as per method purpose: preconcentration and/or sample cleanup

✓ Selection of suitable flow-based system for implementation of in-line/on-line sample pretreatment as a front end to detection systems

✓ Characteristic criteria should be evaluated for investigation of analytical performance of preconcentration/separation methods

✓ Flow-based microextraction methods meet green chemical principles and decrease systematic errors (entirely enclosed systems)


Thank you for your attention