Application Bulletin

Metrohm

Von Interesse für: D'intérêt pour: Of interest for:
Allgemeine HPLC-Detektion / Détection HPLC-Général / General HPLC detection

Nr. 128/1 d,f,e

*Elektrochemische Detektion in der HPLC **

Übersicht und Anwendungsbeispiele zur oxidativen Bestimmung von organischen Verbindungen

Einleitung

In unserer Gebrauchsanweisung zum elektrochemischen Detektor (656 Electrochemical Detector) findet der Anwender alle grundlegenden Angaben zur Funktionsweise und Bedienung des Gerätes sowie zur Handhabung der Elektroden. Hinweise auf die Anforderungen, die an das Trennsystem gestellt werden, wie zur Ursache und Behebung von Detektionsstörungen sind auch darin aufgeführt.

Das vorliegende Application-Bulletin soll einerseits einen Überblick über die wichtigsten Stoffklassen und einige Verbindungen vermitteln, die sich oxidativ gut, d.h. mit Nachweisgrenzen im pg-Bereich detektieren lassen, und andererseits anhand einiger Beispiele mögliche Arbeitsbedingungen für Trennung und elektrochemische Detektion illustrieren.

HPLC-Apparatur mit elektrochemischer Detektion

Der elektrochemische Detektor kann grundsätzlich an alle HPLC-Systeme angeschlossen werden. Für ein erfolgreiches Arbeiten, besonders im pg-Bereich, müssen alle Teile wie Pumpe, Dämpfungssystem, Injektor, Trennsäule, Kapillaren, Kupplungsteile u.a. in einwandfreiem Zustand sein. Bei der elektrochemischen Detektion können auch Störungen durch HPLC-Teile auftreten, die sich z.B. bei der UV-Detektion nicht bemerkbar machten. Es handelt sich dabei oft um Verunreinigungen aus dem System (Trennsäule) oder um Druckschwankungen durch undichte Verbindungsteile.
Auf die folgenden Punkte sei noch besonders hingewiesen:

- **Sauberkeit**
  Es gelten die allgemeinen Anforderungen, die an Arbeiten in der Spurenanalytik gestellt werden. Jede Möglichkeit zur Korrosion von Stahlteilen ist zu vermeiden, da Eisen-II-Ionen stören können. Der Eluent sollte auch keine Komplexbildner enthalten.

- **Pulsation**
  Das Rauschen des Detektorsignals ist von Druckschwankungen in der Zelle abhängig. Ein eventuell zusätzlicher Pulsationsdämpfer basierend auf dem Bourdonrohr-Prinzip hat sich bewährt und ist auch bei Verwendung von Einkolbenpumpen ausreichend.

- **Eluent**
  Viele in der HPLC übliche Lösungsmittel können zur Herstellung des Eluenten verwendet werden.
  Die zur Detektion notwendige Leitfähigkeit wird durch Zugabe eines Leitsalzes erreicht, im allgemeinen genügen Konzentrationen von 1...10 g/L, was eine Leitfähigkeit von ca. 1...10 mS/cm ergibt. Es können u.a. verwendet werden: Sulfate, Nitrate, Phosphate, Essigsäure, Schwefelsäure, Perchlorsäure, Lithiumperchlorat.
  Chloride und Hydroxicarbonsäuren sollten nicht, ionenpaarbildende Reagenzien können z.T. verwendet werden.
  Lösungsmittelgradienten können in den weniger empfindlichen Bereichen gefahren werden (Strombereiche grösser als 100 nA). Der Eluent wird wie üblich am Vakuum oder durch Einleiten von Helium entgast.

- **Stationäre Phase**
  Es können grundsätzlich alle Reversed-Phase- und Ionenaustauschermaterialien verwendet werden.

- **Injektionslösung**
  Wenn immer möglich sollte dafür als Lösungsmittel der Eluent verwendet werden, um Störungen durch Milieuänderungen minimal zu halten (möglichst kleiner Frontpeak).
  Von der Aktivsubstanz sollten geringe Mengen verwendet werden, im allgemeinen weniger als 100 ng.

* Hochdruck-Flüssigkeitschromatographie

*Détection électrochimique en HPLC **

Généralités et exemples d'application de la détermination oxidative des composés organiques.

Introduction

Dans le mode d'emploi METROHM du Détecteur électrochimique 656, l'utilisateur trouve toutes les données fondamentales concernant le fonctionnement et la manipulation de l'appareil ainsi que le traitement des électrodes. Il contient aussi les prescriptions concernant le système de séparation et des conseils sur les causes et l'élimination des perturbations de la détection.

Le présent bulletin d'application est destiné d'une part à donner une vue d'ensemble des principales classes de substances ainsi que quelques composés facilement détectables par les méthodes oxydantes, c'est-à-dire avec des limites de détection dans la gamme pg et d'autre part à illustrer les conditions de travail possibles pour la séparation et la détection électrochimique à l'aide de quelques exemples.

Appareillage HPLC avec détection électrochimique

En principe, le Détecteur électrochimique peut être utilisé avec tous les systèmes HPLC. Pour obtenir de bons résultats, particulièrement dans la gamme pg, tous les composants tels que la pompe, le système d'atténuation, l'injecteur, la colonne de séparation, les capillaires, les tubes de connexion etc. doivent être en parfait état. Dans la détection électrochimique, des perturbations dues aux composants du système HPLC peuvent se manifester, alors qu'elles passeraient inaperçues dans la détection UV. Ces perturbations sont souvent imputable à la contamination venant du système (colonne de séparation) ou aux variations de pression dues à l'étanchéité du tube de connexion.

Il faut porter une attention particulière aux points suivants:

- **Propreté**
  Il convient de prendre les précautions habituelles pour l'analyse des traces, d'éviter la corrosion des parties en acier, puisque les ions ferreux (Fe^{2+}) peuvent fausser les résultats. L'éluant ne doit contenir aucune substance susceptible de former des complexes.

- **Pulsations**
  Il se peut que le bruit du signal du Détecteur provienne des variations de pression dans la cellule détectrice. Un affaiblisseur d'impulsions supplémentaire fonctionnant selon le principe du tube Bourdon s'est montré efficace dans de tels cas et fonctionne adéquatement, même lorsqu'une pompe à un seul cylindre est utilisée.

- **Eluant**
  La plupart des solvants utilisés en HPLC sont utilisables pour préparer l'éluant. On obtient la conductivité supplémentaire requise pour la détection électrochimique en ajoutant un sel "de conductivité", généralement en concentrations de 1...10 g/L, ce qui donne des conductivités d'environ 1...10 mS/cm. On peut utiliser par exemple les solvants suivants: sulfates, nitrates, phosphates, acide acétique, acide sulfurique, acide perchlorique, et perchlorate de lithium.
  Il faut éviter les chlorures et les acides carboxyliques alors que les réactifs formant des paires d'ions peuvent s'utiliser dans certains cas.
  Des gradients peuvent être maîtrisés dans les gammes de faible sensibilité (gammes de courant supérieures à 100 nA).
  L'éluant est dégazé de la façon habituelle ou par passage à travers un courant d'hélium.

- **Phase stationnaire**
  En principe, tous les matériaux à phase inversée ou échange d'ions peuvent être utilisés.

- **Solution d'injection**
  Utiliser si possible l'éluant comme solvant pour éviter des perturbations dues aux modifications de l'environnement (pics frontales les plus basses possibles).

* Chromatographie en phase liquide à haute pression

*Electrochemical detection in HPLC **

General considerations and application examples of the oxidative determination of organic compounds

Introduction

The Operating Instructions for the METROHM 656 Electrochemical Detector provide the user with all basic information concerning the functioning and operation of the instrument, together with treatment of the electrodes. Hints on separating system requirements and on the causes and cures of detection disturbances are also to be found therein.

The present Application Bulletin is intended on the one hand to give a synoptic view of the most important classes of substances and compounds which can be well detected by oxidative methods, i.e. with detection limits in the pg (picogramme, 10^{-12} g) range, and on the other hand to illustrate the possible working conditions for separation and electrochemical detection by means of some examples.

HPLC apparatus with electrochemical detection

In principle, the 656 Electrochemical Detector can be used in conjunction with all HPLC systems.

For successful results, particularly in the pg range, all components, such as pump, dampening system, injector, column, capillaries, tube fittings, etc., must be in perfect condition. With electrochemical detection, disturbances due to components of the HPLC system which would pass unnoticed with UV detection may be recorded. These are often due to contamination from the system (column) or to pressure fluctuations due to imperfect fittings.

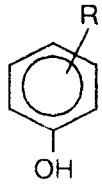
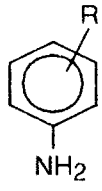
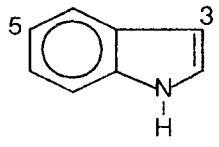
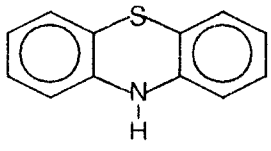
Particular attention should be paid to the following points:

- **Cleanliness**
  The usual precautions for trace analysis should be observed. All corrosion of steel parts should be avoided, since ferrous (Fe^{2+}) ions can interfere. Eluents should not contain complex-forming substances.
- **Pulsation**
  "Noise" in the detector signal may be produced by pressure fluctuations in the detector cell. An additional pulse dampener working on the principle of the Bourdon tube has shown itself to be of value in such cases, and functions quite adequately even where a single-piston pump is used.
- **Eluent**
  Many of the solvents normally used for HPLC can be used in making up the eluent. The extra conductivity required for electrochemical detection is obtained by adding an "electrolyte", generally in concentrations from 1...10 g/L, giving conductivities of about 1...10 mS/cm. Among others, the following can be used: sulfates, nitrates, phosphates, acetic acid, sulfuric acid, perchloric acid and lithium perchlorate.
  Chlorides and hydroxyacids should be avoided, while ion-pairing reagents can be used in certain cases.
  Gradient elution is possible in the upper sensitivity ranges (current ranges greater than 100 nA).
  The eluent is degassed in the usual manner by vacuum or by passing through helium.
- **Stationary phase**
  In principle, all reversed-phase and ion-exchange materials can be used.
- **Injection solution**
  Wherever possible, the eluent should be used as a solvent to avoid disturbances due to change of the environment (lowest possible solvent front).
  The mass of solute injected should not exceed 100 ng in order to avoid surface filming of the electrode.

* High-Pressure Liquid Chromatography

*Electrochemical detection in HPLC **

Important substance-classes

			Voltage U_{pol} *
1.	**Aromatic alcohols**		
1.1	Phenols		+ 1200 mV
1.2	Halogenated Phenols		+ 1200 mV
1.3	Hydroxyphenyls		+ 800 mV
1.4	Catechols		+ 800 mV
1.5	Methoxyphenols		+ 800 mV
1.6	Hydroxycoumarins		+ 1000 mV
1.7	Flavones		+ 1000 mV
1.8	Estrogens		+ 1000 mV
1.9	Tocopherols		+ 800 mV
1.10	Antioxidants		+ 800/1000/1200 mV
1.11	Others		
2.	**Aromatic amines**		
2.1	Anilines		+ 1000 mV
2.2	Benzidines		+ 600 mV
2.3	Sulfonamides		+ 1200 mV
3.	**Indoles**		
3.1	Indolyl-3-derivatives		+ 1000 mV
3.2	5-Hydroxy-indoles		+ 800 mV
4.	**Phenothiazines**		+ 1000 mV
5.	**Mercaptans**	R – SH	+ 800 mV
6.	**Others**		
6.1	Ascorbic acid		+ 800 mV
6.2	Vitamin A		+ 1000 mV
6.3	Carotenes		+ 800 mV
6.4	Purines		+ 800 1000 mV

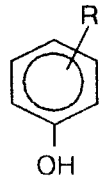
* approximative values

*Electrochemical detection in HPLC **

Some examples of compounds belonging to the substance-classes

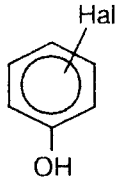
1. Aromatic alcohols

1.1 Phenols



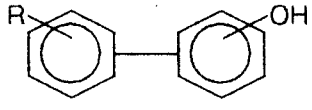
4-Hydroxybenzoic acid
4-Hydroximandelic acid
Hydroxyephedrine
Phenylephrine
Salicylic acids
Synephrin
Tyrosine
Tyramine
Thyroxine
Thyronine
2-Phenylphenol

1.2 Halogenated Phenols



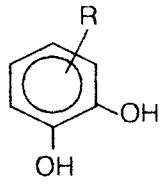
Chlorophenols

1.3 Hydroxybiphenyls



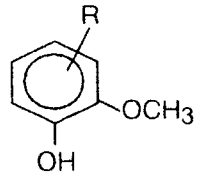
Chlorinated Hydroxybiphenyls

1.4 Catechols



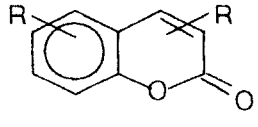
Adrenaline/Noradrenaline
Caffeic acid
Chlorogenic acid
Dopamine
L-Dopa
Gentisic acids
Homogentisic acids
Dihydroxymandelic acid
Isoprenaline
Isoproterenol
Terbutaline

1.5 Methoxyphenols



Homovanillic acid
Metanephrine
3-Methoxy-4-hydroxyphenyl-ethyleneglycol
Normethanephrine
Vanillin
Vanillic acid
Vanillylmandelic acid
α-Methyldopa, -dopamine

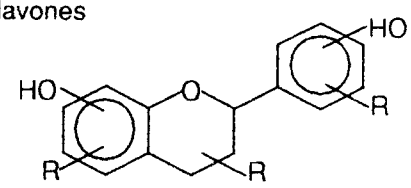
1.6 Hydroxycoumarins



Esculetin
Esculin
Scopoletin
Dicoumarol

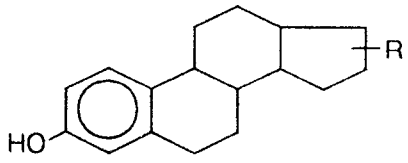
*Electrochemical detection in HPLC **

1.7 Flavones



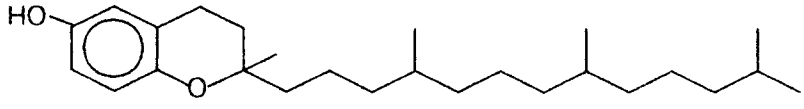
Quercetin
Catechins
Cyanidine
Hesperitin
Rutin

1.8 Estrogens



Estrone
Estradiol
Estriol
Ethynylestradiol
Diethylstilbestrol
Stilboestrol
Dienestrol
Hexestrol
Zearalenon
Zeranol

1.9 Tocopherols



α-, β-, γ-, δ-Tocopherols
Tocol

1.10 Antioxidants

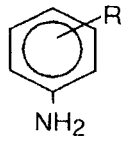
tert-Butylhydroxyanisoles (BHA)
Butylphenols
tert-Butylhydroxytoluenes (BHT)
Gallates
Nordihydroguaiaretic acid (NDGA)
tert-Butylhydroxyquinones (TBHQ)

1.11 Others

Morphine
Dihydromorphine
Apomorphine
Tetrahydrocannabinols
Cinchonine
8-Hydroxycarteolol
Salsolinol
β-Cetotetrine
Folic acid

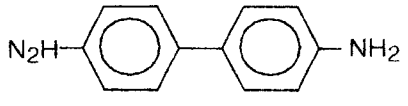
2. Aromatic amines

2.1 Anilines



Chloroanilines
Bromoanilines
Dichloroanilines
Toluidines
Amino-chlorophenols
p-Phenylenediamine

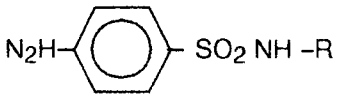
2.2 Benzidines



Benzidine
substituted Benzidines

*Electrochemical detection in HPLC **

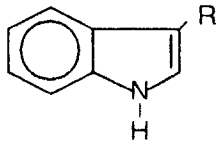
2.3 Sulfonamides



Sulfanilamide
Sulfamethoxydiazine
and others

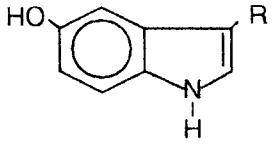
3. Indoles

3.1 Indolyl-3-derivatives



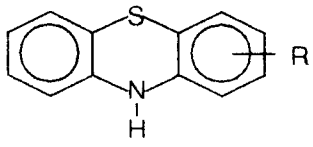
Indolyl-3-acetic acid
3-Methylindole
Tryptophan
Tryptamine
Melatonin

3.2 5-Hydroxyindoles



Serotonin
5-Hydroxyindolylacetic acid
5-Hydroxytryptophan

4. Phenothiazines



Chloropromazine
Promethazine
Perphenazine
and others

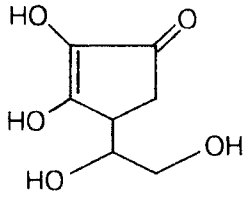
5. Mercaptans

R - SH

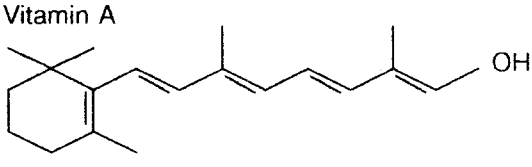
Cysteine
Penicillamine
Glutathione

6. Others

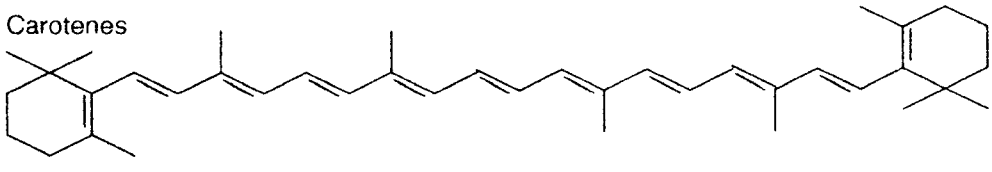
6.1 Ascorbic acid



6.2 Vitamin A

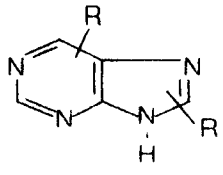


6.3 Carotenes



α-Carotene
β-Carotene
Lycopene
Phytoene
Xanthophyll

6.4 Purines



Uric acid
Xanthine
Guanine

*Electrochemical detection in HPLC **

Instrumentation

METROHM ELCD System:
641 VA Detector
656 Electrochemical Detector
Auxiliary electrode (AE) : Glassy carbon
Reference electrode: Silver/silver chloride
c(KCl) = 3 mol/L

HPLC System:
Pump: Altex Mod. 110
Pulse dampening system: Bourdon tube principle
Lit.: Ventura et al., Anal. Chem. 50, 1017 (1978)
Loop injection volume: 20 µL
Temperature: 20 °C

Names and symbols

Volume fraction of substance B: $V_B/\Sigma_i V_i$	Φ_B
Mass concentration: m_i/V	ρ_i
Volume	V
Glassy carbon	GC

List of the single compounds in the chromatograms

Compound	substance-class	chromatogram/page
Acetaminophen	1.1	10
L-Adrenaline	1.4	13/15
Ascorbic acid	6.1	29/30
3-tert-Butylhydroxyanisole	1.10	21
tert-Butylhydroxytoluene	1.10	22
β-Carotene	6.3	31/32
Catechin	1.7	17
3-Chloroaniline	2.1	23
Chlorpromazine	4.	26
o-Cresol	1.1	11
m-Cresol	1.1	11
p-Cresol	1.1	11
L-Cysteine	5.	27
L-Dopa	1.4	13
Dopamine	1.4	13/14/15
3,4-Dihydroxyphenylacectic acid	1.4	25
2,5-Dimethylphenol	1.1	11/12
3,5-Dimethylphenol	1.1	11

*Electrochemical detection in HPLC **

Compound	substance-class	chromatogram/page
Esculin	1.6	16
Estradiol	1.8	19
Estriol	1.8	19
Estrone	1.8	19
L-Glutathione	5.	27
Homovanillic acid	1.5	25
L-Noradrenaline	1.4	13/14/15
Nordihydroguaiaretic acid	1.10	21
Penicillamine	5.	27/28
Pentachlorophenol	1.2	12
Phenol	1.1	11/12
p-Phenylenediamine	2.1	23
4-Phenylphenol	1.1	12
Propyl gallate	1.10	21
Rutin	1.7	17
Quercetin	1.7	18
Sulfanilamide	2.3	24
Sulfamethoxydiazine	2.3	24
2,4,5-Trichlorophenol	1.2	12
2,3,4,5-Tetrachlorophenol	1.2	12
α-Tocopherol	1.9	20/31/32
β-, γ-Tocopherol	1.9	20
δ-Tocopherol	1.9	20
o-Toluidine	2.1	24
D,L-Tryptophan	3.1	25
Uric acid	6.4	29
Vitamine-A-acetate	6.2	31

HPLC/ELCD-Application

Substance ***Phenols***

Acetaminophen in serum,
$\rho = 200$ ng/mL

Liquid Chromatographic System

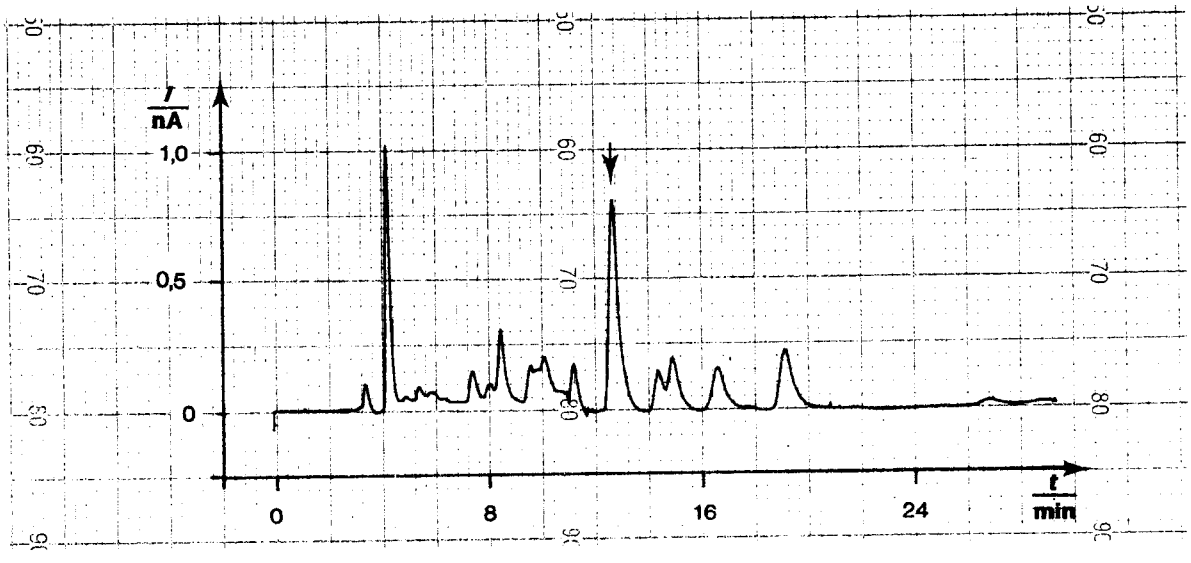
Column:	LiChrosorb RP-18, 5 µm, 250 mm × 4.6 mm ID
Eluent:	methanol-water (140:18), with ammonium acetate and acetic acid $\phi(MeOH) = 0.885$, $\rho(CH_3COONH_4) = 5.2$ g/L, $\rho(HAc) = 7$ g/L (pH 5.2)
Flow rate:	0.8 mL/min
Injection volume:	20 µL

Detector

Working electrode:	GC
Voltage:	+800 mV
Range:	5 nA

Sample serum, $V = 200$ µL, extracted with dichlormethane-2-propanol-ether

Literature D.J. Miner et al.
J. Pharm. Sci. 68, 96 (1979)



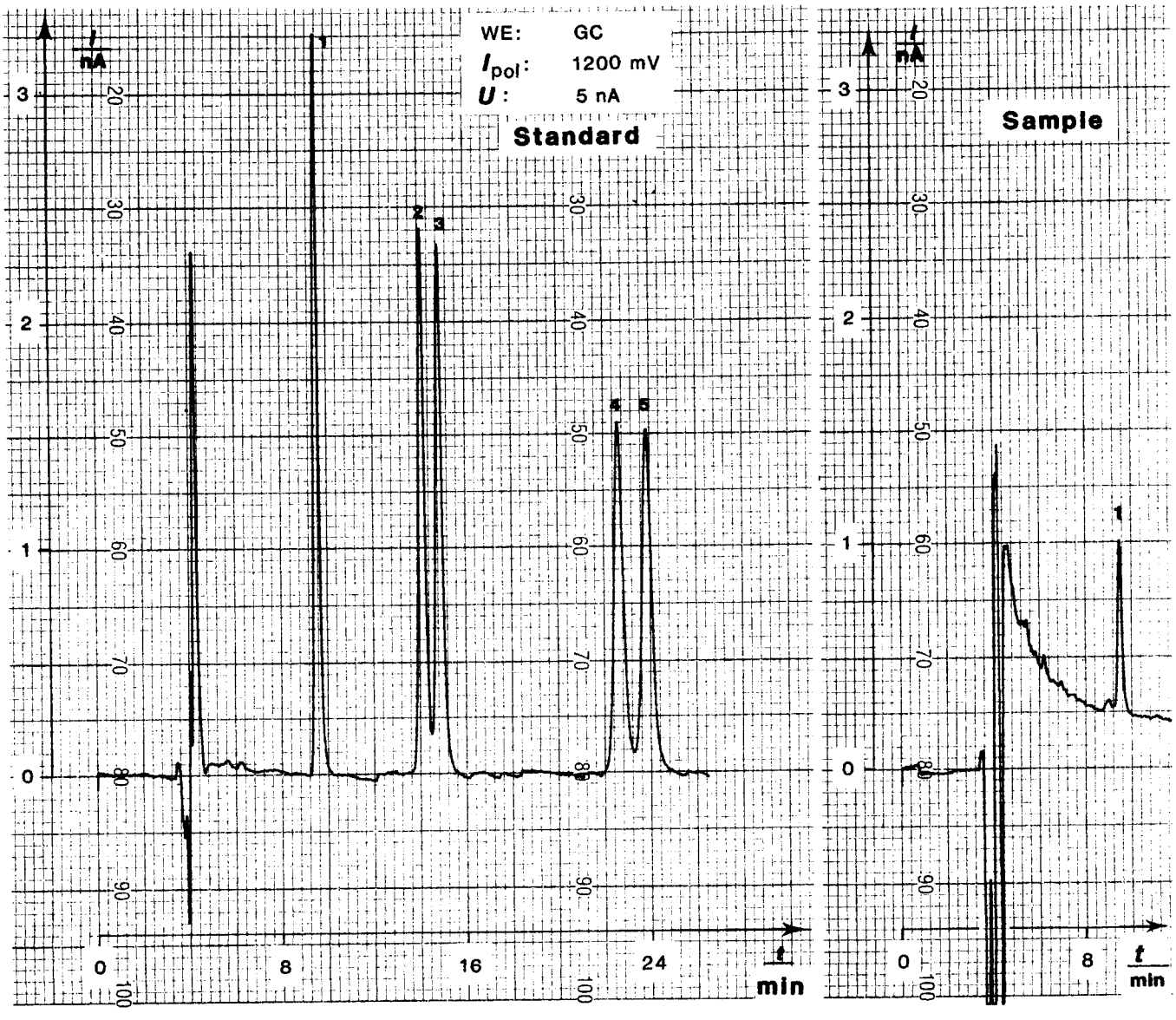
HPLC/ELCD-Application

Substance *Phenols in wastewater*

1 Phenol
2 p-, m-Cresol
3 o-Cresol
4 3,5-Dimethylphenol
5 2,5-Dimethylphenol

Liquid Chromatographic System

Column:	Nucleosil 5 C_{18}, 5 µm, 250 mm × 4.6 mm ID
Eluent:	methanol-water (1:1), with potassium nitrate and sulfuric acid ϕ(MeOH) = 0.5, $\rho(KNO_3)$ = 2 g·L, $\rho(H_2SO_4)$ = 0.05 g·L
Flow rate:	0.7 mL/min
Mass of solute injected:	standard: 1 ng each sample: filtrated wastewater, ρ(Phenol) = 10 µg/L



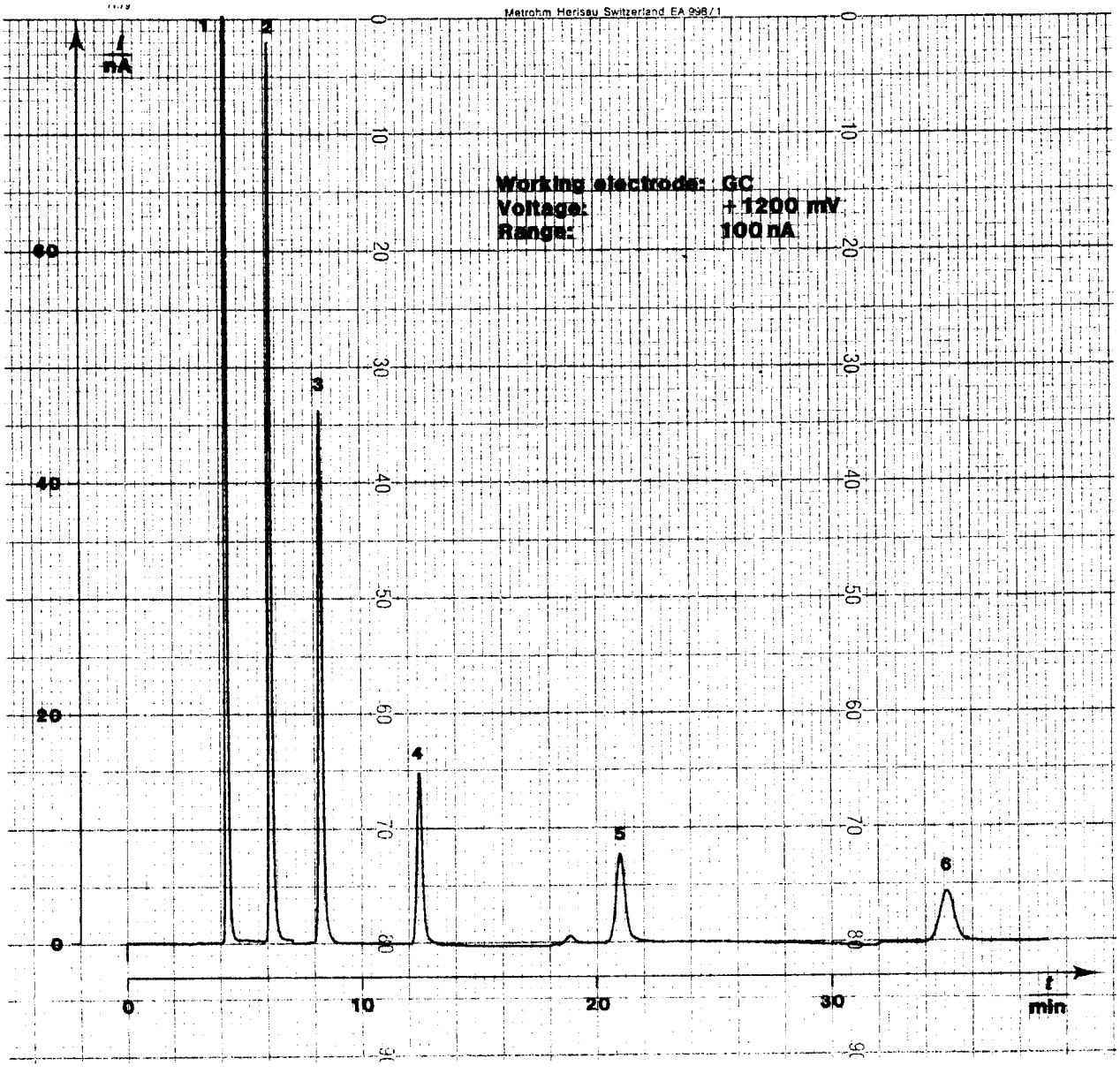
HPLC/ELCD-Application

Substance ***Halogenated Phenols***

1 Phenol
2 2,5-Dimethylphenol
3 4-Phenylphenol
4 2,4,5-Trichlorophenol
5 2,3,4,5-Tetrachlorophenol
6 Pentachlorophenol

Liquid Chromatographic System

Column:	Nucleosil 5 C_{18}, 5 µm, 250 mm × 4.6 mm ID
Eluent:	methanol-water (7:3), with potassium nitrate and sulfuric acid ϕ(MeOH) = 0.7, $\rho(KNO_3)$ = 2 g/L, $\rho(H_2SO_4)$ = 0.05 g/L
Flow rate:	0.9 mL/min
Mass of solute injected:	20 ng each



HPLC/ELCD-Application

Substance *Catecholamine*

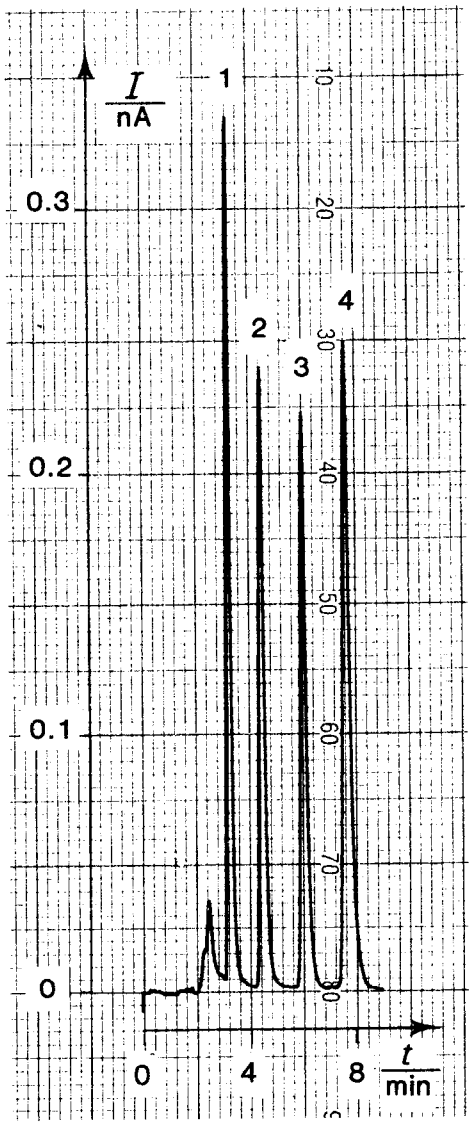
1 L-Noradrenaline
2 L-Adrenaline
3 L-Dopa
4 Dopamine-HCl

Liquid Chromatographic System

Column:	LiChrosorb RP-18, 5 µm, 250 mm × 4.6 mm ID
Eluent:	water, with ammonium sulfate and acetic acid (pH 3.2) $\rho((NH_4)_2SO_4) = 5$ g/L, $\rho(HAc) = 3$ g/L
Flow rate:	1 mL/min
Mass of solute injected:	200 pg each

Detector

Working electrode:	GC
Voltage:	+ 800 mV
Range:	0.5 nA



HPLC/ELCD-Application

Substance *Catecholamines from tissue*

1 L-Noradrenaline $m = 15$ pg
2 Dopamine $m = 40$ pg

Liquid Chromatographic System

Column:	Nucleosil 5 SA, 5 µm, 150 mm × 4.6 mm ID
Eluent:	acetate-citrate-buffer, pH 5.2
Flow rate:	1 mL min
Injection volume:	20 µL

Detector

Working electrode:	GC
Voltage:	+ 800 mV
Range:	0.5 nA

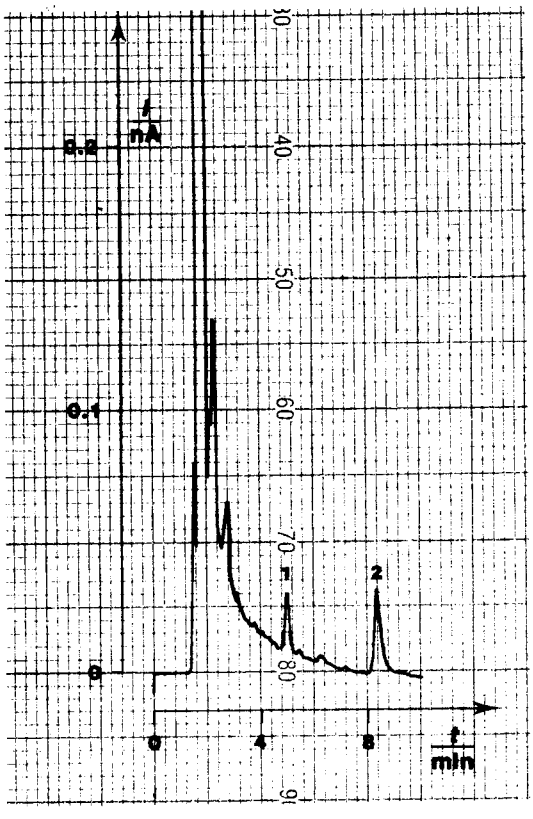
Sample preparation

Isolation of Catecholamines from tissue by alumina (Al_2O_3) extraction

Literature

R. Keller et al.
Life Sci. 19, 995 (1976)

S. Allemark et al.
J. Liq. Chrom. 2, 277 (1979)



HPLC/ELCD-Application

Substance *Catecholamines from urine*

1 L-Noradrenaline (NA)
2 L-Adrenaline (A)
3 Dopamine (DA)

Liquid Chromatographic System

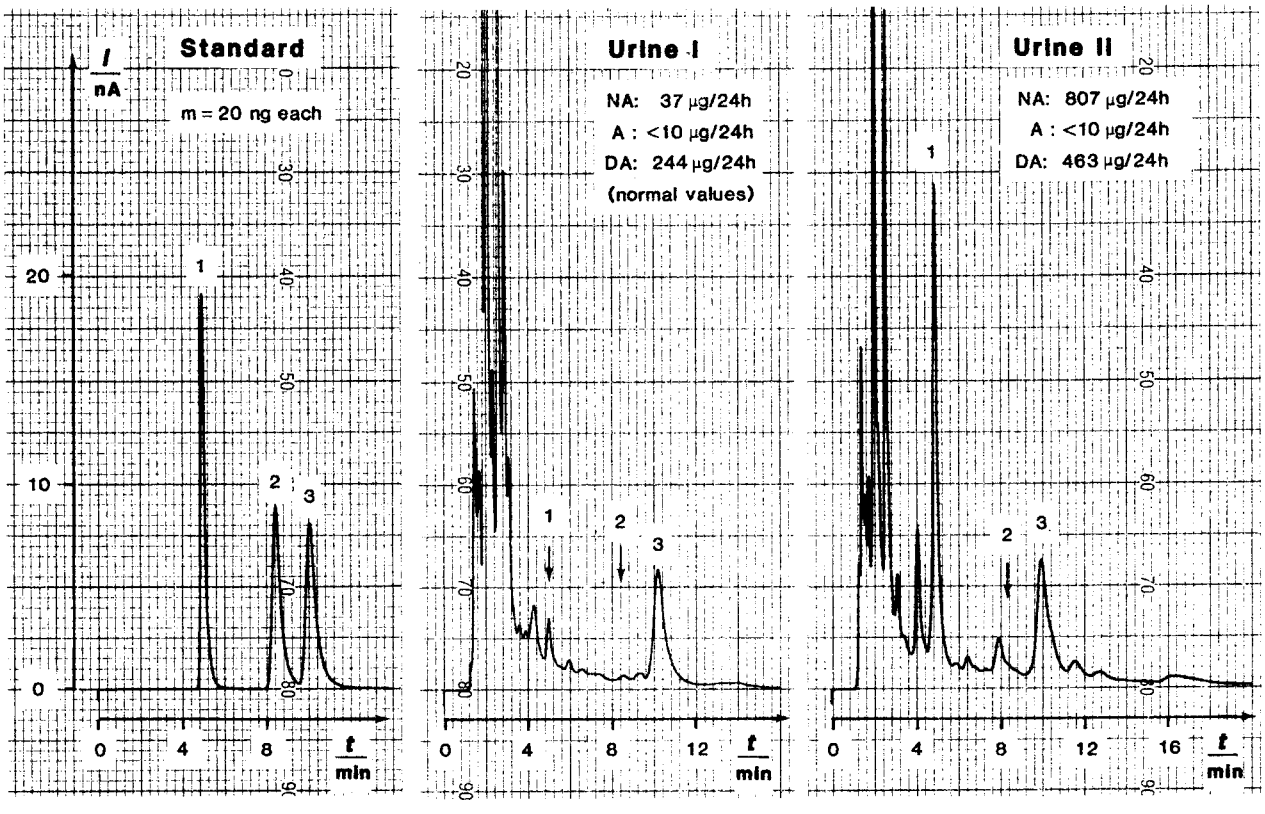
Column:	Nucleosil 10 SA, 10 µm, 250 mm × 4.6 mm ID
Eluent:	water, with sodium acetate and sodium citrate, pH 4.2
Flow rate:	2 mL/min
Injection volume:	20 µL

Detector

Working electrode:	GC
Voltage:	+ 800 mV
Range:	50 nA

Sample preparation

Isolation of catecholamines from urines ($V = 5$ mL) by alumina (Al_2O_3) extraction



HPLC/ELCD-Application

Substance *Hydroxycoumarins*

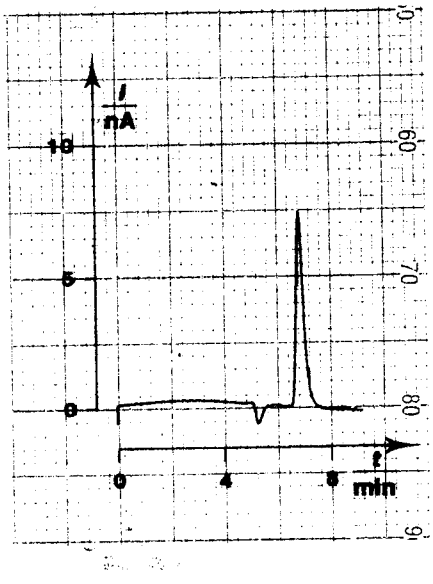
Esculin

Liquid Chromatographic System

Column:	LiChrosorb RP-18, 5 µm, 250 mm × 4.6 mm ID
Eluent:	methanol-water (2:3), with acetic and lithium perchlorate ϕ(MeOH) = 0.4, ρ(HAc) = 5 g/L, $\rho(LiClO_4)$ = 5 g/L
Flow rate:	0.5 mL·min
Mass of solute injected:	20 ng

Detector

Working electrode:	GC
Voltage:	+ 1000 mV
Range:	50 nA



HPLC/ELCD-Application

Substance *Flavones*

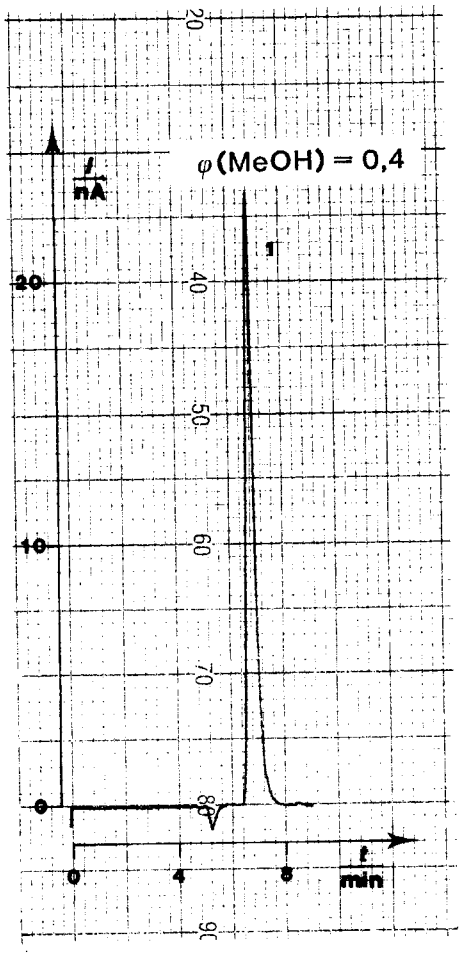
1 Catechin
2 Rutin

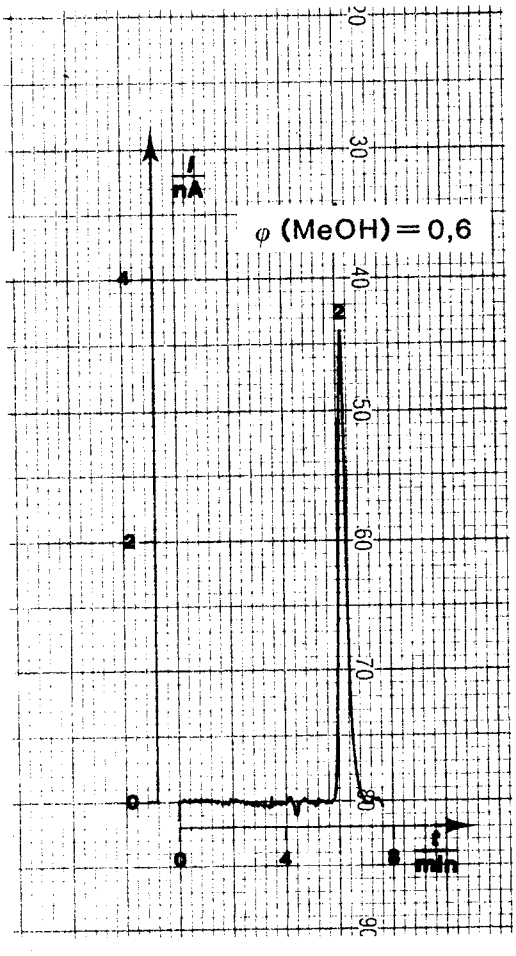
Liquid Chromatographic System

Column:	LiChrosorb RP-18, 5 µm, 250 mm × 4.6 mm ID
Eluent:	methanol-water, with lithium perchlorate and acetic acid $\rho(LiClO_4) = 5$ g L, $\rho(HAc) = 5$ g L
Flow rate:	0.5 mL/min
Mass of solute injected:	20 ng each

Detector

Working electrode:	GC
Voltage:	+ 1000 mV
Range:	50 nA or 10 nA





HPLC/ELCD-Application

Substance ***Flavones***

Quercetin
from hawthorn (crataegus oxyacanthae)
tincture

Liquid Chromatographic System

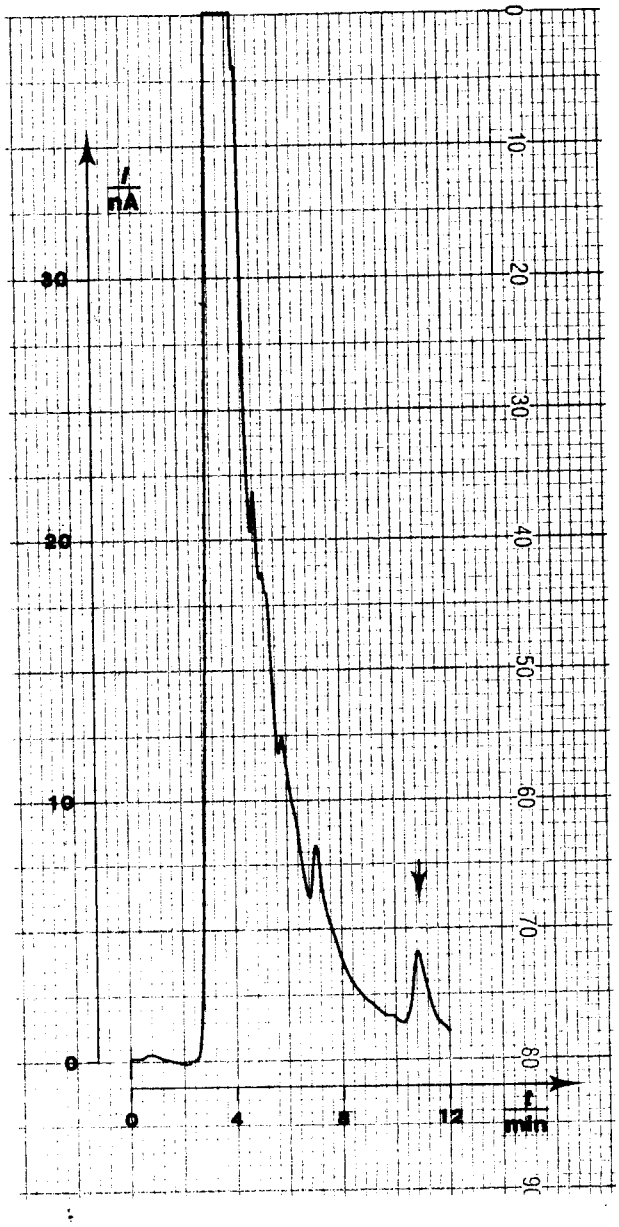
Column:	LiChrosorb RP-2, 5 µm, 250 mm × 4.6 mm ID
Eluent:	methanol-water (1:1), with lithium perchlorate and acetic acid ϕ(MeOH) = 0.5, $\rho(LiClO_4)$ = 2 g/L, ρ(HAc) = 10 g/L
Flow rate:	0.9 mL min
Injection volume:	20 µL

Detector

Working electrode:	GC
Voltage:	+ 1000 mV
Range:	50 nA

Sample preparation

tincture diluted with eluent
ϕ(tincture) = 0.1



HPLC/ELCD-Application

Substance *Estrogens*

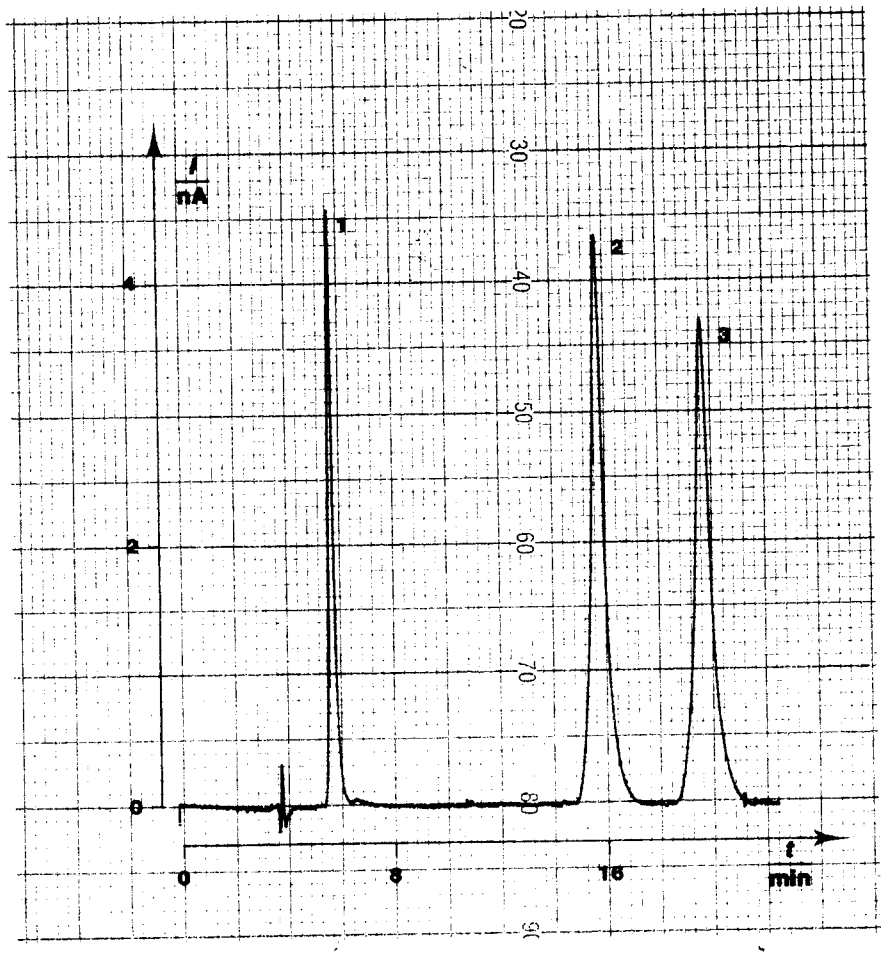
1 Estriol
2 Estradiol
3 Estrone

Liquid Chromatographic System

Column:	LiChrosorb RP-18, 5 µm, 250 mm × 4.6 mm ID
Eluent:	acetone–water (1:1), with lithium perchlorate and acetic acid ϕ(acetone) = 0.5, $\rho(LiClO_4)$ = 5 g/L, ρ(HAc) = 5 g/L
Flow rate:	0.7 mL/min
Mass of solute injected:	20 ng each

Detector

Working electrode:	GC
Voltage:	+ 1000 mV
Range:	10 nA



HPLC/ELCD-Application

Substance *Tocopherols from soya oil*

1 δ-Tocopherol
2 β, γ-Tocopherol
3 α-Tocopherol

Liquid Chromatographic System

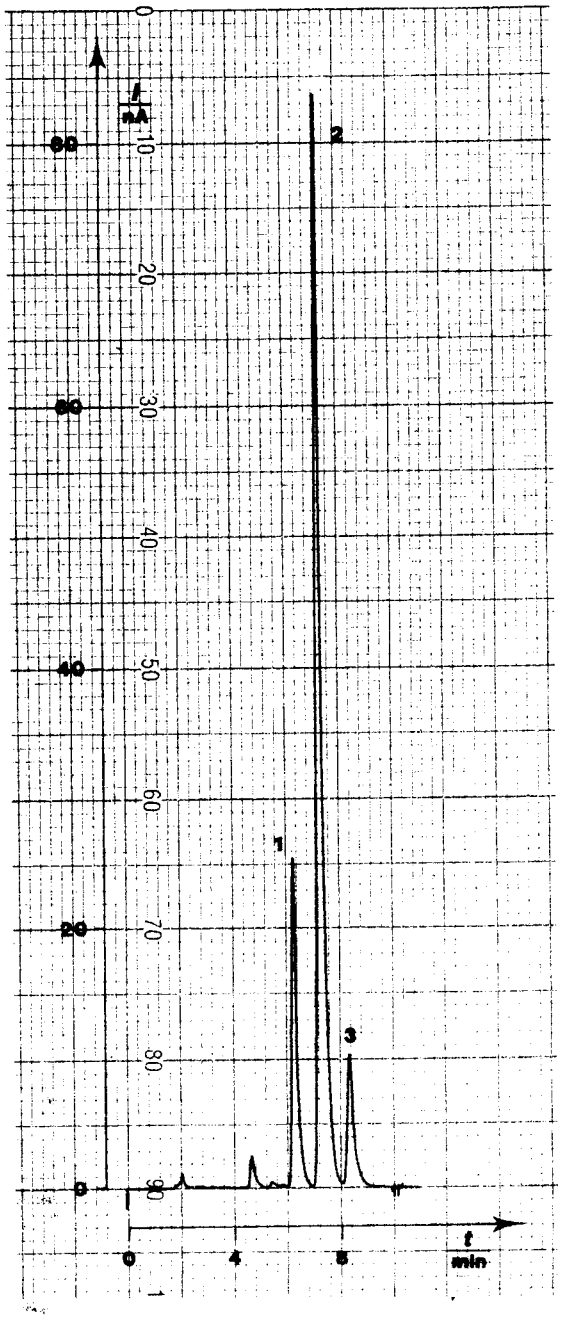
Column:	LiChrosorb RP-18, 5 µm, 250 mm × 4.6 mm ID
Eluent:	methanol, with lithium perchlorate and acetic acid $\rho(LiClO_4) = 5$ g/L, $\rho(HAc) = 1$ g/L
Flow rate:	1.5 mL/min
Injection volume:	20 µL

Detector

Working electrode:	GC
Voltage:	+800 mV
Range:	100 nA

Sample preparation

soya oil $m = 182$ mg
extracted with eluent $V = 20$ mL



HPLC/ELCD-Application

Substance ***Antioxidants***

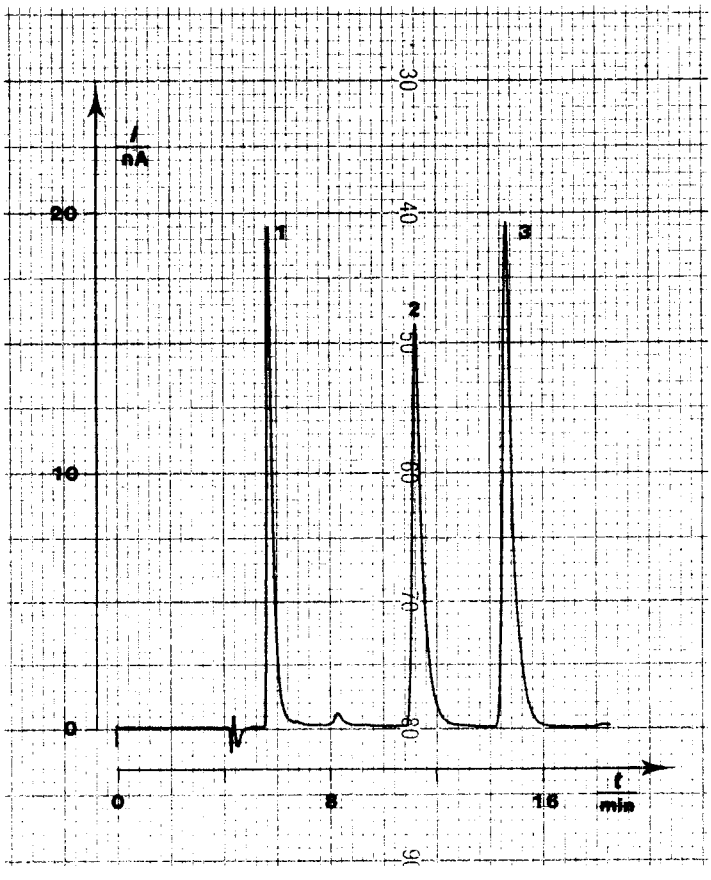
1 Propyl gallate
2 Nordihydroguaiaretic acid
3 3-tert-Butyl-4-hydroxyanisole

Liquid Chromatographic System

Column:	LiChrosorb RP-18, 5 µm, 250 mm × 4.6 mm ID
Eluent:	methanol-water (7:3), with lithium perchlorate and acetic acid $\phi(MeOH) = 0.7$, $\rho(LiClO_4) = 5$ g/L, $\rho(HAc) = 5$ g/L
Flow rate:	0.6 mL/min
Mass of solute injected:	20 ng each

Detector

Working electrode:	GC
Voltage:	+800 mV
Range:	50 nA



HPLC/ELCD-Application

Substance ***Antioxidants***

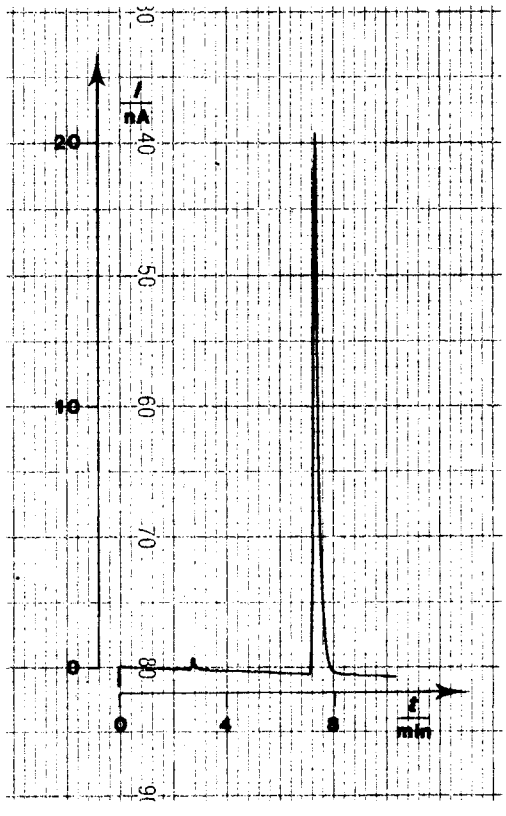
tert-Butylhydroxytoluene

Liquid Chromatographic System

Column:	LiChrosorb RP-18, 5 µm, 250 mm × 4.6 mm ID
Eluent:	methanol-water (9:1), with lithium perchlorate and acetic acid ϕ(MeOH) = 0.9, $\rho(LiClO_4)$ = 5 g/L, ρ(HAc) = 5 g/L
Flow rate:	1 mL/min
Mass of solute injected:	20 ng

Detector

Working electrode:	GC
Voltage:	+ 1200 mV
Range:	50 nA



HPLC/ELCD-Application

Substance ***Anilines***

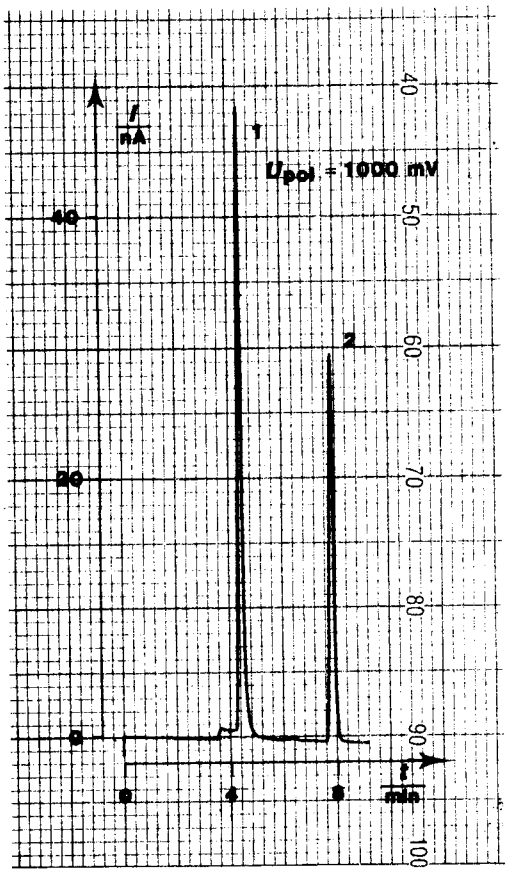
1 p-Phenylenediamine
2 3-Chloroaniline

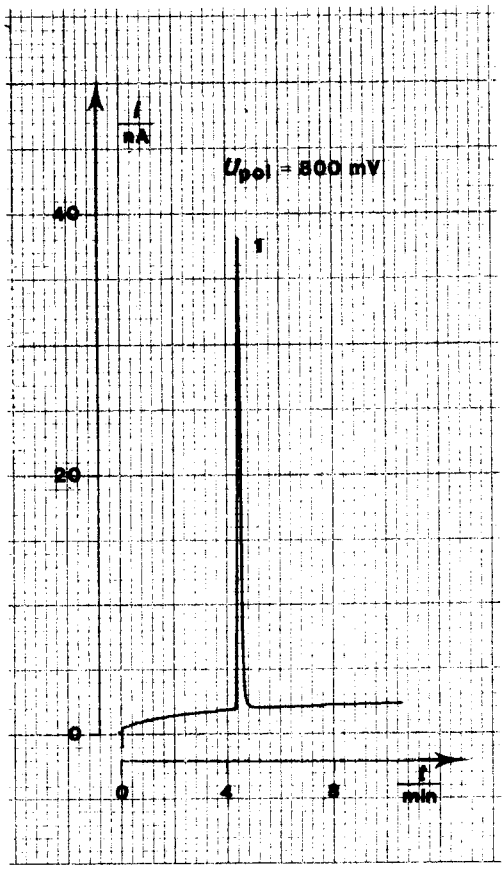
Liquid Chromatographic System

Column:	Nucleosil 5 C_{18}, 5 µm, 250 mm × 4.6 mm ID
Eluent:	methanol-water (1:1), with potassium nitrate and sulfuric acid ϕ(MeOH) = 0.5, $\rho(KNO_3)$ = 2 g/L, $\rho(H_2SO_4)$ = 0.05 g/L
Flow rate:	0.7 mL/min
Mass of solute injected:	1: 20 ng, 2: 25 ng

Detector

Working electrode:	GC
Voltage:	+ 1000 mV
Range:	100 nA





HPLC/ELCD-Application

Substance

Anilines
Sulfonamides

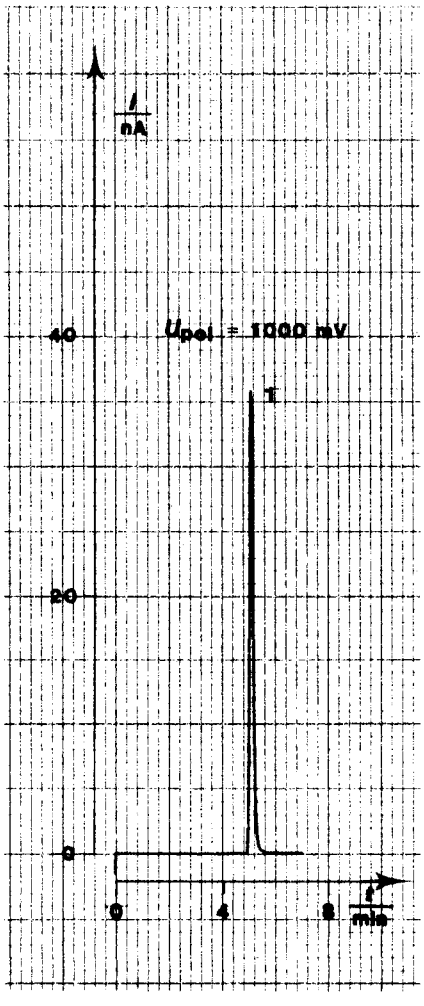
1 o-Toluidine
2 Sulfanilamide
3 Sulfamethoxydiazine

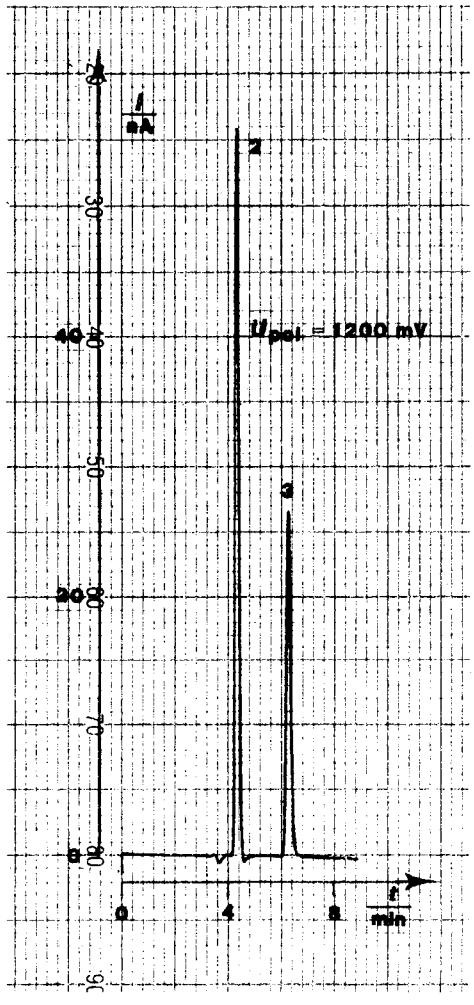
Liquid Chromatographic System

Column:	Nucleosil 5 C_{18}, 5 µm, 250 mm × 4.6 mm ID
Eluent:	methanol-water (1:1), with potassium nitrate and sulfuric acid ϕ(MeOH) = 0.5, $\rho(KNO_3)$ = 2 g/L, $\rho(H_2SO_4)$ = 0.05 g/L
Flow rate:	0.7 mL/min
Mass of solute injected:	20 ng each

Detector

Working electrode:	GC
Voltage:	+ 1000 mV / 1200 mV
Range:	100 nA





HPLC/ELCD-Application

Substance

Indoles
Aromatic alcohols

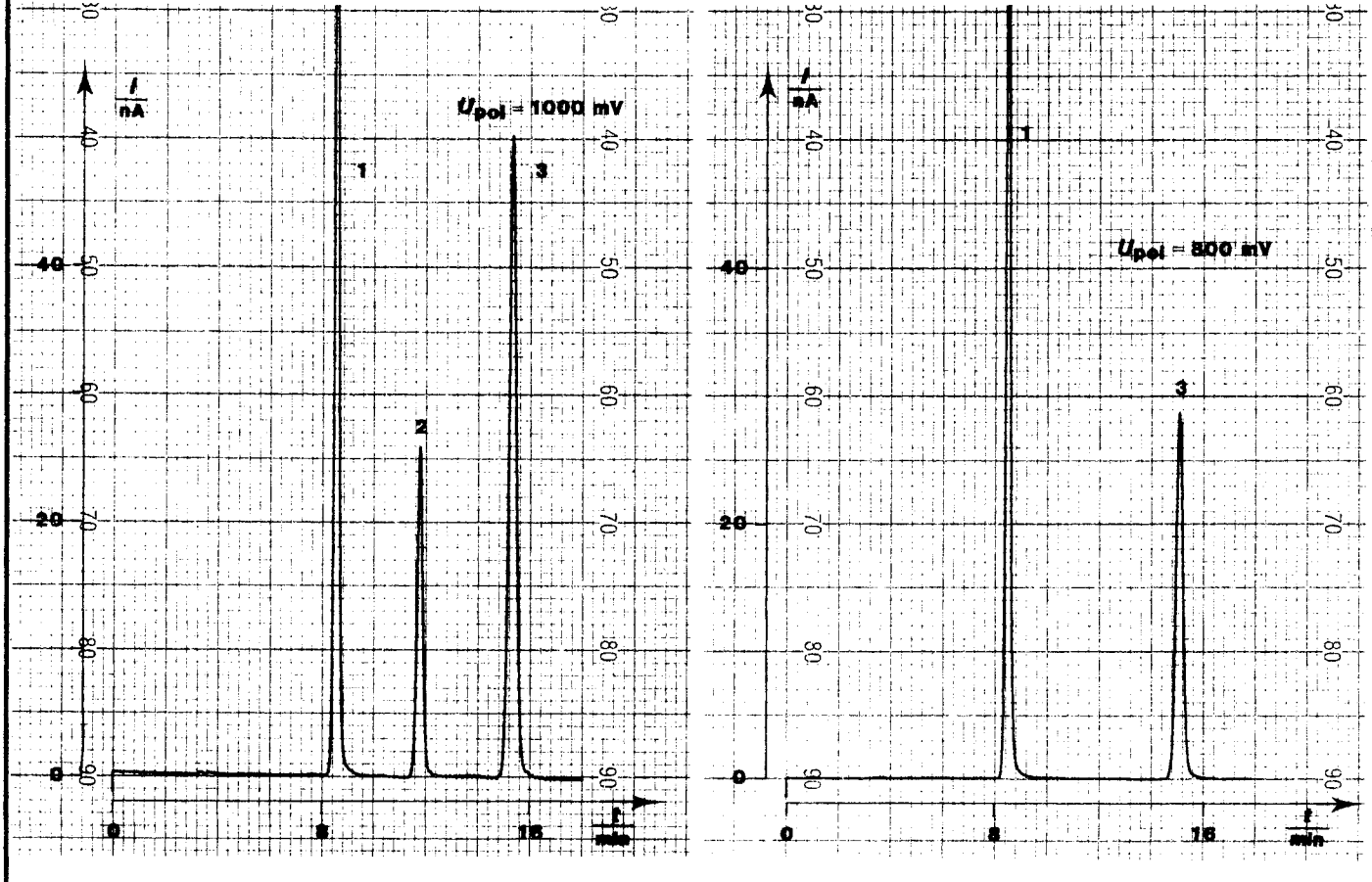
1 3,4-Dihydroxyphenylacetic acid
2 D,L-Tryptophan
3 Homovanillic acid

Liquid Chromatographic System

Column:	Nucleosil 5 C_{18}, 5 µm, 250 mm × 4.6 mm ID
Eluent:	methanol-water (1:1), with potassium nitrate and sulfuric acid ϕ(MeOH) = 0.3, $\rho(CH_3COONH_4)$ = 6 g/L, $\rho(H_2SO_4)$ = 1.5 g/L
Flow rate:	0.7 mL/min
Mass of solute injected:	40 ng each

Detector

Working electrode:	GC
Voltage:	+ 1000 mV / 800 mV
Range:	100 nA



HPLC/ELCD-Application

Substance *Phenothiazines*

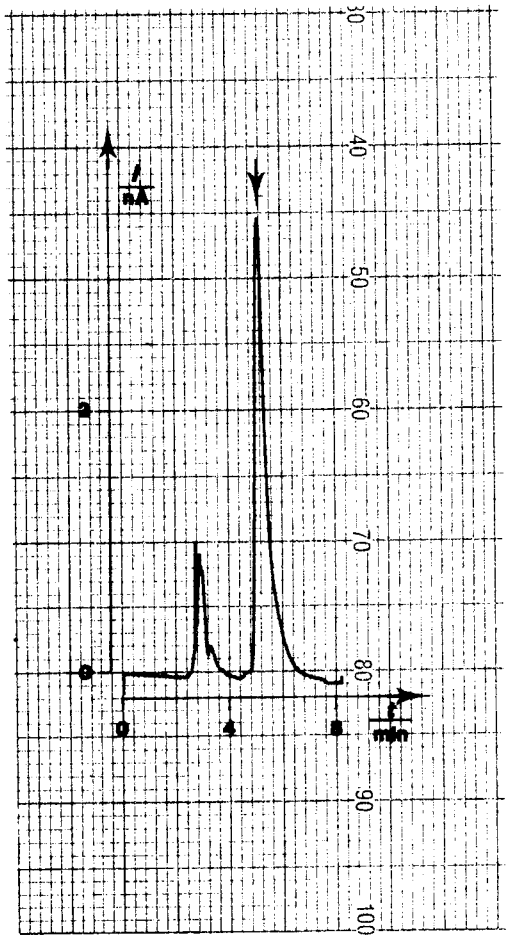
Chlorpromazine-HCl

Liquid Chromatographic System

Column:	LiChrosorb RP-2, 5 µm, 250 mm × 4.6 mm ID
Eluent:	methanol-water (65:35), with lithium perchlorate and acetic acid $\phi(MeOH) = 0.65$, $\rho(LiClO_4) = 2$ g L, $\rho(HAc) = 1$ g/L
Flow rate:	1 mL/min
Mass of solute injected:	20 ng

Detector

Working electrode:	GC
Voltage:	+ 1000 mV
Range:	10 nA



HPLC/ELCD-Application

Substance *Mercaptans*

1 L-Cysteine
2 L-Glutathione
3 Penicillamine

Liquid Chromatographic System

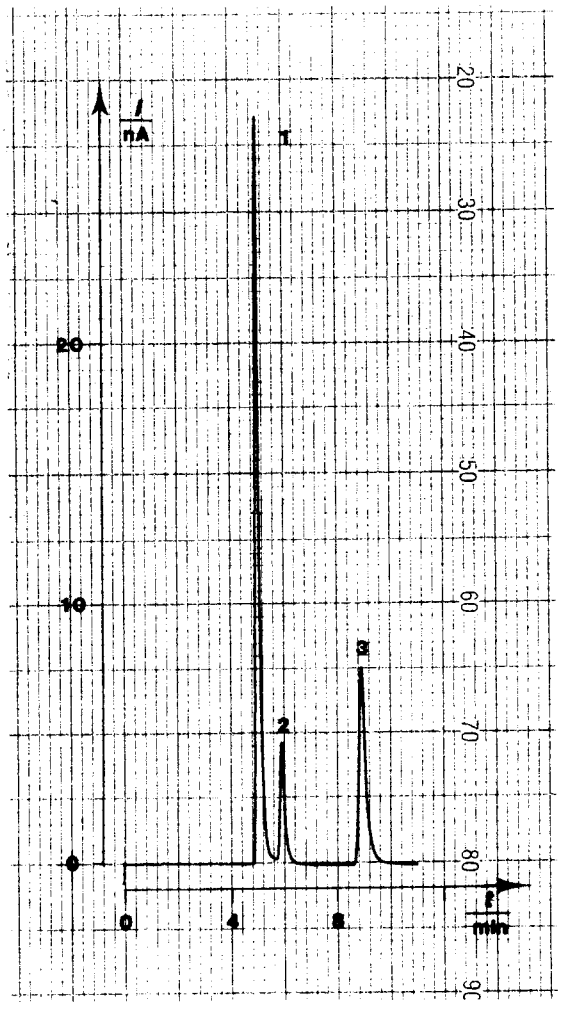
Column:	Nucleosil 5 SA, 5 µm, 150 mm × 4.6 mm ID
Eluent:	water, with ammonium citrate and phosphoric acid $\rho(C_6H_6O_7(NH_4)_2 \cdot H_2O) = 4.5$ g/L, $\rho(H_3PO_4) = 6$ g/L
Flow rate:	1 mL/min
Mass of solute injected:	20 ng each

Detector

Working electrode:	Au
Voltage:	+ 800 mV
Range:	50 nA

Literature

D.L. Rabenstein and R. Saetre
Anal. Chem. 49, 1036 (1977)



HPLC/ELCD-Application

Substance ***Mercaptans***

Penicillamine from urine,
ρ = 20 µg/mL

Liquid Chromatographic System

Column:	Nucleosil 5 SA, 5 µm, 150 mm × 4.6 mm ID
Eluent:	water, with ammonium citrate and phosphoric acid, pH 2.2 $\rho(C_6H_6O_7(NH_4)_2 \cdot H_2O) = 4.5$ g/L, $\rho(H_3PO_4) = 6$ g/L
Flow rate:	1 mL/min
Injection volume:	20 µL

Detector

Working electrode:	Au
Voltage:	+ 800 mV
Range:	100 nA

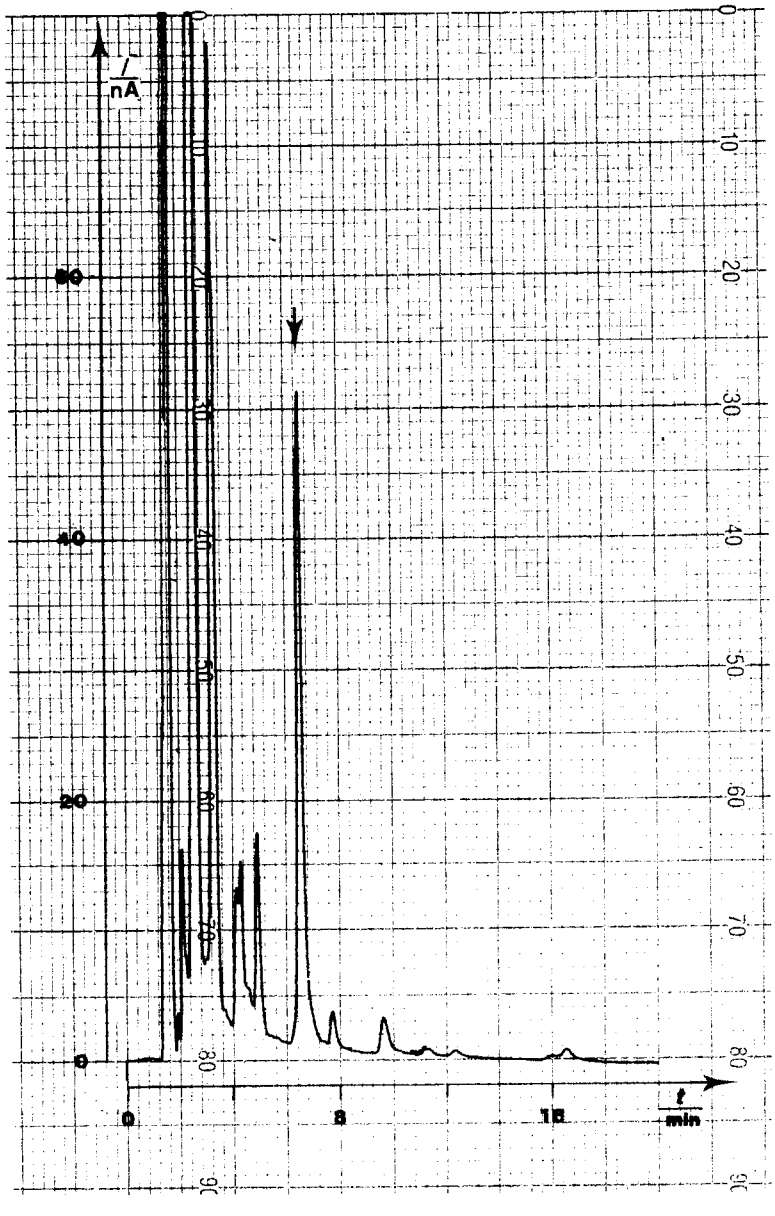
Sample

urine diluted with eluent
ϕ(urine) = 0.1

Literature

D.L. Rabenstein and R. Saetre
Anal. Chem. 49, 1036 (1977)

F. Kreuzig
private communication



HPLC/ELCD-Application

Substance *Vitamins*
Purines

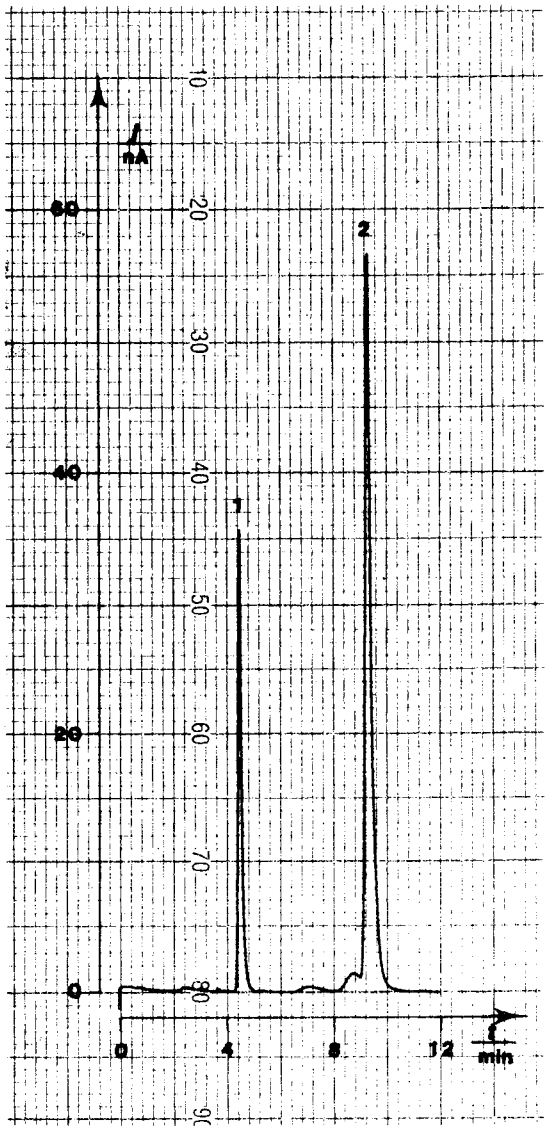
1 Ascorbic acid
2 Uric acid from urine or serum

Liquid Chromatographic System

Column:	LiChrosorb RP-18, 5 µm, 250 mm × 4.6 mm ID
Eluent:	water, with meta-phosphoric acid $\rho(HPO_3) = 8$ g/L
Flow rate:	1 mL/min
Injection volume:	20 µL

Detector

Working electrode:	GC
Voltage:	+ 800 mV
Range:	100 nA



Sample preparation

urine: diluted with eluent
ϕ(urine) = 0.0025

serum: deproteinised serum
diluted with eluent
ϕ(serum) = 0.1

Literature

E.S. Wagner et al.
J. Chrom. 163, 225 (1979)

HPLC/ELCD-Application

Substance *Vitamins*

Ascorbic acid
from fruit drinks, fruit juices,
pharmaceutical preparations

Liquid Chromatographic System

Column:	LiChrosorb RP-18, 5 µm, 250 mm × 4.6 mm ID
Eluent:	water, with meta-phosphoric acid $\rho(HPO_3) = 8$ g/L
Flow rate:	1 mL/min
Injection volume:	20 µL

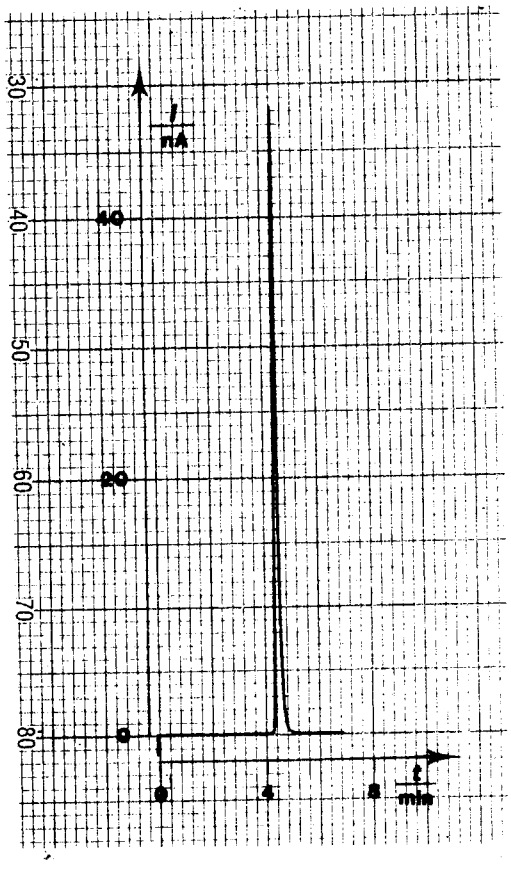
Detector

Working electrode:	GC
Voltage:	+ 800 mV
Range:	100 nA

Literature

E.S. Wagner et al.
J. Chrom. 163, 225 (1979)

L.A. Pachla et al.
Methods in Enzymology 62, 15 (1979)



HPLC/ELCD-Application

Substance *Vitamins*

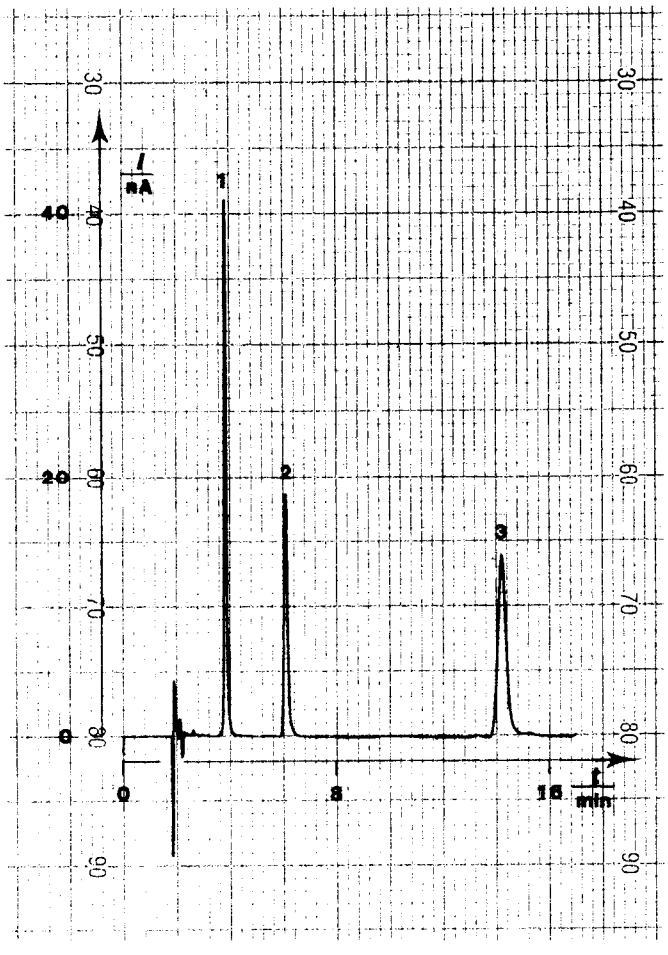
1 Vitamin-A-acetate
2 α-Tocopherol
3 β-Carotene

Liquid Chromatographic System

Column:	Nucleosil 5 C_{18}, 5 µm, 250 mm × 4.6 mm ID
Eluent:	methanol, with lithium perchlorate and acetic acid $\rho(LiClO_4) = 2$ g/L, $\rho(HAc) = 1$ g/L
Flow rate:	1.5 mL min
Mass of solute injected:	20 ng each

Detector

Working electrode:	GC
Voltage:	+ 1000 mV
Range:	100 nA



HPLC/ELCD-Application

Substance *Carotene*
Tocopherol

1 α-Tocopherol
2 β-Carotene
in carrot juice

Liquid Chromatographic System

Column:	Nucleosil 5 C_{18}, 5 µm, 250 mm × 4.6 mm ID
Eluent:	methanol, with lithium perchlorate and acetic acid $\rho(LiClO_4) = 2$ g L, $\rho(HAc) = 1$ g L
Flow rate:	1.5 mL/min
Mass of solute injected:	standard: 1: 4 ng, 2: 8 ng

Sample preparation extraction with methanol

Detector

Working electrode:	GC
Voltage:	+ 800 mV
Range:	10 nA