Stopped-flow fluorimetric determination of theophylline in pharmaceutical preparations. M[a]. C. Gutiérrez, A. Gómez-Hens and D. Pérez-Bendito.

The reaction of theophylline with cerium(IV) has been applied to the kinetic-fluorimetric determination of theophilline using a simple and versatile modular stopped-flow system. The method is based on the monitoring of the variation in the fluorescence intensity during the formation of a 1:1 compound between the oxidation product of theophilline and cerium(III). The stopped-flow technique provides the data required to determine the reaction rate from each kinetic curve in only 8 s. The linear range of the proposed method is 1–250 μg ml^{-1} of theophylline and the detection limit is 0.95 μg ml^{-1}. The stopped-flow method has been satisfactorily applied to the determination of theophylline in various pharmaceutical preparations. Analytical recoveries are in the range 95.0–102.0%. – Analyst **113**, 559–562 (1988). Dept. Anal. Chem., Fac. Sci., Univ., Córdoba (E)

Determination of reserpine and hydrochlorothiazide in commercial tablets by liquid chromatography with fluorescence and UV absorption detectors in series. U.R. Cieri.

A procedure is presented for the determination of reserpine and hydrochlorothiazide in commercial tablets by LC. Reference and sample solutions are prepared in methanol. For LC, a normal phase column is used, methanol is the eluting solvent, and 2 detectors are arranged in series. A fluorescence detector set at an excitation wavelength of 280 nm and emission wavelength of 360 nm quantitates reserpine, and a UV absorption detector set at 345 nm determines hydrochlorothiazide. Several synthetic mixtures and commercial tablets containing the 2 ingredients were analyzed by the proposed method. – J. Assoc. Off. Anal. Chem. **71**, 515–518 (1988). Food Drug Admin., Philadelphia, PA (USA)

Determination of the evaporation rate of essential oils and perfumery compositions using gas chromatography. T.A. Rudolfi, M.M. Shchedrina and L.O. Mindlin.

Die Methode zur Bestimmung der Verdampfungsrate von ätherischen Ölen und Parfümgemischen basiert auf der GC mit Temperaturprogrammierung und ist vergleichbar der simulierten Destillation. Es wird eine kurze Capillarsäule benutzt, die jedoch nicht mit einer flüssigen Phase beschichtet ist. Die Ergebnisse werden dargestellt als Beziehung zwischen Säulentemperatur und dem prozentualen Anteil der verdampften Probe. – Chromatographia **25**, 520–522 (1988). All-Union Res. Inst. Natural Synthetic Odour Substances, Moscow (SU) M.J. Rittich

Flavor characterization of dentifrices using kinetic headspace gas chromatography. E.A. Tavss, S.G. Wiet, R.S. Robinson, J. Santalucia and D.L. Carroll.

A new instrumental technique, kinetic headspace gas chromatography (KHS-GC), was developed to measure relative quantity and composition of flavor released from dentifrices into the headspace of sealed vials, before equilibrium was attained. Dentifrice formulations varying in composition or processing were analyzed for volatile flavor components, providing a quantitative instrumental determination of the effect of these factors on flavor. High correlations were found between the results of the KHS-GC procedure and human perceived flavor attributes of dentifrices as determined by trained sensory panelists. This method has applications in a wide array of product categories, such as foods, beverages and personal care products. – J. Chromatogr. **438**, 273–280 (1988). Colgate-Palmolive Comp., Res. Developm. Div., Piscataway, NJ (USA)

Stability-indicating liquid chromatographic determination of alpha-ionone in toothpaste. R.J. Trivedi.

A simple, sensitive, and rapid liquid chromatographic method for quantitating α-ionone in toothpaste at levels of 20 ppm in the presence of large amounts of flavor has been developed. 10 μm C_{18} packing and methanol/acetonitril/0,01 M K_2HPO_4 (35:20:45) as mobile phase are used. The method is accurate, precise, cost-effective, and specific for a α-ionone. Average recovery of a laboratory-prepared sample was 99.0% with the relative standard deviation was 1.29% (n = 6). – J. Assoc. Off. Anal. Chem. **71**, 36–37 (1988). Warner-Lambert, Consum. Prod. Res. Dev. Cent., Morris Plains, NJ (USA)

Determination of thioglycolic acid, dithiodiglycolic acid, cysteine and cystine in hair-waving solutions by HPLC. J. Koyama, T. Matsumoto, Y. Ohtsu and O. Nakata.

A rapid and simple method by using HPLC was developed for the determination of thioglycolic acid, dithiodiglycolic acid, cysteine and cystine in hair-waving solutions. Ion pair method was suitable for the separation of these ingredients. HPLC conditions used for the separation were: column packing and size, CAPCELL PAK C_{18} and 4.6 mm i.d. × 250 mm; mobile phase, a mixture of 5% acetonitrile-95% water containing 0.1% phosphoric acid and 3.5 mM 1-heptanesulfonic acid; detection, UV 210 nm. Recoveries of these four ingredients from the model sample were 100 ~ 102%. *p*-Hydroxybenzoic acid (0.25 mg/5 ml) was added to 0.25 g of sample as an internal standard and then it was diluted to ca. 40 ml with water; 10 μl of this sample solution was injected into chromatograph. By using this method, the four ingredients in 9 commercial hair-waving solutions were accurately determined without interferences of other ingredients. – Bunseki Kagaku **37**, 142–146 (1988) (Japanisch, mit engl. Zus.fass.). Shiseido Toxicol. Anal. Res. Center, Yokohama-shi, Kanagawa (J)

A method for the determination of N-nitrosoalkanolamines in cosmetics. H. Sommer and G. Eisenbrand.

Die N-Nitrosoalkanolamine werden säulenchromatographisch abgetrennt, silyliert und die TMS-Derivate gaschromatographisch analysiert. – *Arbeitsweise.* 2 g Probe in 9 ml Wasser lösen, 100 μl NEPHA als innerer Standard und 0,5 g Ammoniumsulfamat zufügen und mit Natriumchlorid sättigen. Bei stabiler Emulsionsbildung pH mit NaOH auf 8,5–9,5 einstellen, 1,5 ml Chloroform zufügen und Mischung über eine Kieselgursäule geben, mit 3 ml Wasser nachspülen, äquilibrieren (20 min), mit 100 ml Cyclohexan/Dichlormethan (1:1) auswaschen und mit 150 ml n-Butanol eluieren. Unter steigendem Vakuum im Rotationsverdampfer bei 40°C abdampfen, Rückstand in 20 ml Chloroform/Aceton (5:1) aufnehmen, über eine Kieselgelsäule geben und mit dem Lösunggsmittel bis zu 80 ml Gesamtvolumen nachspülen. Mit 50 ml Aceton eluieren, im Rotationsverdampfer auf 5 ml einengen und mit Aceton in einen Kolben überspülen: Lösungsmittel im Stickstoffstrom entfernen. *Silylierung.* Rückstand mit 150 μl MSHFBA versetzen und nach 45 min mit i-Octan zu 0,5 ml ergänzen. Externer Standard: 100 ng NDELA plus NEPHA; Lösungsmittel unter Stickstoff entfernen und derivatisieren. *GC.* App. Hewlett Packard 5880 A mit TEA; 3 m × 2 mm-Säule (silanisiertes Glas) mit 6% OV 275 an Volaspher A 2. Trägergas Helium (20 ml/min), angewandte Menge 5 μl. Injektionstemperatur 200°C, Temperaturprogrammierung 150°C (1 min), dann bis 170°C (2°C/min), bis 220°C (10°C/min); Pyrolysetemperatur 400–500°C. Wiederauffindung innerer Standard 95%, Erfassungsgrenze 5 μg/kg. – Z. Lebensm. Unters. Forsch. **186**, 235–238 (1988). Fachrichtg. Lebensm.chem. u. Umwelttoxik., Fachber. Chem., Univ., Kaiserslautern (D) D. Rittweger

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3 BIOCHEMICAL AND CLINICAL ANALYSIS

Pressurized microwave digestion of biological samples for metal determination. I. Kojima, T. Uchida and C. Iida.

The utility of microwave oven for simple and rapid acid digestion of zoological and botanical materials was investigated by using a double Teflon vessel with a polypropylene jacket. The microwave-assisted digestion of samples with the HNO_3-HCl-$HClO_4$-HF mixture in the bomb offers the advantage of taking much shorter process for sample preparation. The digestion was completed within 15 min even under a very mild condition. Eight elements (Cu, Fe, Mn, Zn, Ca, Mg, K and Na) in the standard reference materials were determined by one-drop flame atomic absorption and emission spectrometry. Good agreement with the certified values was obtained. – Anal. Sci. **4**, 211–214 (1988). Lab. Anal. Chem., Dept. Appl. Chem., Nagoya Inst. Technol., Nagoya (J)

Transient response of the two-dimensional glucose sensor. J.Y. Lucisano and D.A. Gough.

The two-dimensional enzyme electrode has certain advantages for the development of an implantable glucose sensor, but details of the dynamic response of this electrode have not been previously understood. A model and experimental observations are presented that demonstrate the effects of substrate concentration, mass-transfer parameters, immobilized enzyme activity, geometry, and sensor size. With appropriate design, the transient response can be rapid and comparable to that of the one-dimensional sensor. – Anal. Chem. **60,** 1272–1281 (1988). Dept. Appl. Mechan. Engin. Sci., Bioengin. Gr., Univ. California, San Diego, La Jolla, CA (USA)

Alcohol-FET sensor based on a complex cell mebrane enzyme system. E. Tamiya, I. Karube, Y. Kitagawa, M. Ameyama and K. Nakashima.

Verff. beschreiben den Aufbau und die Herstellung eines FET-Sensors (Feld-Effekt-Transistor) zur Alkoholbestimmung. Dazu wird ein in eine Zellmembran eingebettetes komplexes Enzymsystem und ein ionenselektiver Feld-Effekt-Transistor (ISFET) verwendet. Die dafür geeignete Zellmembran gewinnt man aus *Gluconobacter suboxydans* IFO 12528. Diese wandelt durch ihr inneres Enzymsystem Ethanol zu Essigsäure um. Man immobilisiert die Membran an der ISFET-Öffnung mit Hilfe von Calciumalginatgel, das mit Nitrocellulose überzogen wird. Die Messungen erfolgen in der Weise, daß man ein so hergestelltes ISFET (1), ein Bezugs-ISFET ohne Zellmembran (ISFET 2) und eine Ag/AgCl-Bezugselektrode in 5 mM Tris-Malatpuffer (pH 5,5; 25°C) eintaucht und die Spannungsdifferenz zwischen ISFET 1 und 2 mißt. Zur Stabilisierung wird Pyrrolochinchinon zugegeben. Responsezeit 10 min. Es besteht eine lineare Beziehung zwischen Spannungsdifferenz und Ethanolkonzentration aufwärts bis 20 mg/l. Das Sensorsystem bleibt ca. 40 h bei einer Temperatur $< 30°C$ stabil. Es spricht an auf Ethanol, Propan-1-ol und Butan-1-ol, nicht aber auf Methanol, Propan-2-ol und Butan-2-ol und eignet sich für Blutethanolbestimmungen. – Anal. Chim. Acta **207,** 77–84 (1988). Res. Lab. Resources Utiliz., Tokyo Inst. Technol., Midori-ku, Yokohama (J) W. Czysz

Direct kinetic method for the determination of phosphate. A.P. van Zanten and J.A. Weber.

Anorganisches Phosphat in Serum läßt sich einfach und genau spektralphotometrisch durch Messung der Anfangsgeschwindigkeit zur Bildung des unreduzierten heteropolysauren Phosphomolybdatkomplexes bei 334 nm bestimmen. Zur Reagensherstellung löst man 42 ml analysenreines H_2SO_4 in 500 ml dest. H_2O. Nach Abkühlung auf Raumtemperatur werden in dieser Lösg. 2,78 g Ammoniummolybdat, 3,0 g Polyvinylpyrrolidon ($M_r = 40000$) und 5 ml Triton X 405 gelöst und auf 1 l mit dest. H_2O verdünnt. Diese Lösg. hält sich bei 2–8°C bis zu einem Monat. Die Konzentration von Polyvinylpyrrolidon wird im Hinblick auf die Bestimmung von anorganischem Phosphat in Serum im Eppendorf EPOS Analysator (Eppendorf Gerätebau, Netheler und Hinz, Hamburg) optimiert. Unter Verwendung von wäßrigen KH_2PO_4-Standardlösg. bis 5,0 mM mit 0–50 g/l an Albumin beobachtet man eine von der Albuminkonzentration unabhängige Linearität bis zu 3,0 mM KH_2PO_4. Bei Konzentrationen von 0,9 und 2,3 mM erreicht man innerhalb eines Analysendurchgangs ($n = 20$) Variationskoeffizienten von 0,96 bzw. 1,35%. Die diesbezüglichen täglichen Ungenauigkeiten belaufen sich auf 1,65 bzw. 2,32% ($n = 20$). Mögliche aus Hyperlipämie, hohem Bilirubin- oder hohem Proteingehalt der Serumproben herrührende Störungen werden erörtert. – J. Clin. Chem. Clin. Biochem. **25,** 515–517 (1987). Dept. Clin. Chem., Mun. Hosp. Slotervaart, Amsterdam (NL) F.T. Bartsch

Eisenbestimmung im Serum (Plasma) mit Tripyridyl-triazin (TPTZ) und Citratpuffer. Bewertung der Methode sowie Anpassung an einen SMAC II und RA-1000. U.B. Seifert, E. Solem und M. Zirker.

Die hier vorgestellte Methode zur Eisenbestimmung im Serum bzw. Plasma zeichnet sich durch eine hohe Spezifität für Eisen aus. Gleichzeitig vorhandene Kupferionen stören nur wenig. An Hämoglobin gebundenes Eisen stört nicht. Die Reaktionsbedingungen sind so gewählt, daß Eisen aus seiner Bindung zum Transferrin gelöst wird, nicht aber aus der Bindung zum Hämoglobin. Außerdem kommt es bei dem verwendeten pH-Wert nicht zu einer Proteinpräzipitation, so daß ohne vorherige Enteiweißung gemessen werden kann. Die Nachweismethode ist kostengünstig im Vergleich zu anderen handelsüblichen Tests. – Lab. Med. **12,** 174–178 (1988). Zentrallab. Zentrum Innere Med., Klinikum Univ., Frankfurt (D) M.J. Rittich

Determination of iron, copper and zinc in a single aliquot of serum sample using 4-[(5-bromo-2-pyridyl)azo]resocinol. G.M. Castillo, R.J. Thibert, N.D. Seudeal and B. Zak.

The individual and the sequential determinations of Fe, Cu, and Zn using 4-[(5-bromo-2-pyridyl)azo]resorcinol (Br-PAR) were investigated in terms of its applicability in serum samples. The methods obey Beer's law from 25–500 μg dl^{-1}. The methods show consistency between individual and sequential determination. The recovery studies at five different levels of Fe, Cu, and Zn averaged out to be (mean ± SD): 99.7 ± 5.1%, 104.4 ± 3.8% and 100.9 ± 3.2%, respectively. The within-assay CV's taken at six different serum levels for Fe, Cu, and Zn averaged out to be 3.4 ± 1.2%, 3.7 ± 1.8%, and 3.5 ± 1.9%, respectively. The between-assay CV's for Fe, Cu, and Zn in serum taken at four different levels and 21 different occasions averaged out to be 4.3 ± 1.0%, 5.3 ± 1.7%, and 4.3 ± 1.0%, respectively. – Microchem. J. **37,** 99–109 (1988). Dept. Chem., Biochem., Univ. Windsor, Ont. (CDN)

A new microchemical approach to amperometric analysis. J. Talbott and J. Jordan.

Cottrell chronoamperometry of metabolites relies on a scheme which incorporates dual specificity safeguards of enzymatic catalysis and controlled potential electrolysis. Relevant methodological principles are illustrated by the selective oxidation of β-D-glucose with benzoquinone. Gluconic acid yields of +99.9% were attained in the presence of glucose oxidase. Concomitantly, equivalent amounts of benzoquinone were reduced to hydroquinone, which conveniently was quantitated in quiescent solutions, at stationary palladium thin-film anodes. Sample sizes of 25 μl were used, documenting microclinical applicability. – Microchem. J. **37,** 5–12 (1988). Dept. Chem. 152 Davey Lab., State Univ., University Park, PA (USA)

Electrochemical behavior of small biological molecules at organic donor-acceptor electrodes in aqueous media. K. McKenna, S.E. Boyette and A. Brajter-Toth.

Verff. untersuchten das elektrochemische Verhalten von NADH, Xanthin, Harnsäure, 6-Mercaptopurin, 6-Thioxanthin, Dopamin und Ascorbinsäure an organometallartigen Polymerpaste-Elektroden. Das neue Elektrodenmaterial enthält als wirksames Substrat Tetrathiafulvalin- und N-Methylphenazin-tetracyanochinodimethan (TTF-TCNQ und NMP-TCNQ). Die Herstellung dieser Komplexe und ihre Verarbeitung in den Elektrodenkörper wird beschrieben. Die weiteren Versuche zeigen, daß die Reaktivität der zitierten kleineren biologischen Moleküle an dieser Art von Polymerpaste-Elektroden generell geringer ist als an den sehr aktiven Graphitelektroden. Typische Nachweisgrenzen sind ca. 10^{-5} M, typische Obergrenzen der Linearität liegen bei 10^{-3} M. – Anal. Chim. Acta **206,** 75–84 (1988). Dept. Chem., State Univ., Gainesville, FL (USA) W. Czysz

Determination of myo-inositol in a flow-injection system with co-immobilized enzyme reactors and amperometric detection. B. Olsson, G. Marko-Varga, L. Gorton, R. Appelqvist and G. Johansson.

Zur Bestimmung von Myo-Inositol (Hexahydroxycyclohexan) wird ein selektives und empfindliches Fließinjektionsverfahren beschrieben. Man arbeitet mit einem Enzymreaktor, in dem drei Enzyme an porösen Glasperlen gemeinsam immobilisiert sind: Inositol-Dehydrogenase (IDH), Lactat-Dehydrogenase (LDH) und Lactat-Oxidase (LOD). Beim Passieren des Enzymreaktors entsteht unter Mitwirkung von NAD^+ am Ende eine äquivalente Menge H_2O_2, welches in einem zweiten, in das Fließsystem integrierten Reaktor (mit immobilisierter Peroxidase) Hexacyanoferrat(II) zu Hexacyanoferrat(III) umwandelt. Letzteres wird in einem elektrochemischen Durchflußdetektor amperometrisch bei 0 mV vs. GKE nachgewiesen. Das System liefert lineare Anzeigen für Myo-Inositol im Konzentrationsbereich 1–300 μM (injiziertes Lösungsvolumen 25 μl). Maximaler Probendurchsatz 90/h. Der Enzymreaktor erwies sich über mindestens 5 Wochen stabil. – Anal. Chim. Acta **206,** 49–55 (1988). Dept. Anal. Chem., Univ., Lund (S) W. Czysz

Fast-scan voltammetry of biogenic amines. J.E. Baur, E.W. Kristensen, L.J. May, D.J. Wiedemann and R.M. Wightman.

Cyclic voltammograms were recorded at a scan rate of 200 V s^{-1} at carbon-fiber electrodes with and without coating of a perfluorinated ion-exchange material. Voltammograms were recorded in a flow injection apparatus, and background subtraction was used to remove the residual current. Voltammograms for the oxidation of 4-methylcatechol at uncoated electrodes had the peak amplitude expected for the previously published oxidation mechanism for catechols at intermediate pH. In contrast, voltammograms for dopamine, dihydroxybenzylamine, and norepinephrine showed much larger peak currents. Semiintegration of these voltammograms gives a peak-shaped curve indicative of adsorption. At coated electrodes, the voltammograms for anionic species are greatly attenuated, while for the biogenic amines the peak currents are larger than at uncoated electrodes. – Anal. Chem. **60,** 1268–1272 (1988). Dept. Chem., Univ., Bloomington, IN (USA)

Determination of low parts per billion levels of amines in urine by liquid membrane sample cleanup directly coupled to a gas-liquid chromatograph. G. Audunsson.

An automated sample preparation by the liquid membrane technique directly coupled to a gas chromatograph is demonstrated for the determination of amines in urine. The alkalized sample comes into contact with *n*-undecane immobilized in a membrane made of Teflon wherein the amines are extracted. On the opposite side of the membrane a stagnant buffer traps solely those amines which become protonted at the prevailing pH. After the sample passes, the accumulated plug of amines is swept from the membrane separator to an interface which injects a heartcut of the plug into the gas chromatograph. The presented technique lowers the detection limit of the manual gas chromatographic method for pure aqueous solutions by more than 2 orders of magnitude for urine as a matrix, while the availability of sample limits the enrichment obtainable for simpler matrices. The overall repeatability is 3.5–4% for concentrations down to 1 ppb in urine. – Anal. Chem. **60,** 1340–1347 (1988). Dept. Anal. Chem., Univ., Lund (S)

Basal plasma-catecholamine-level determination using HPLC-ED and different sample cleanup techniques. B. Dirks, C. Vorwalter, A. Grünert and F.W. Ahnefeld.

Adrenaline, dopamine and noradrenaline demonstrated good stability during different stages of collection and long-term storage. Using a new electrochemical detector and improving mobile phase parameters, we obtained a detection limit of 2 pg per injection. Good separation of dihydroxyphenylacetic acid was also attained, which is important in investigations with intensive care patients. Good accuracy and precision, demonstrated in the daily quality control measurements taken over a five month period, verified the reliability of the chromatographic separation. However, there was a decrease in the recovery of very low amounts of catecholamines, added to fresh frozen plasma that had previously been made catecholamine-free. According to the widely-accepted extraction method of Anton and Sayre, it is argued that the unknown affinity of catecholamines to acid-prepared aluminium oxide (in comparison to catecholamine-protein binding constants) explains the low accuracy in measurement at very low plasma levels. We thus compared this sample preparation method to recently published extraction procedures. – Chromatographia **25,** 223–228 (1988). Univ. Klinik Anästh., Klinik Univ., Ulm (D)

Simultaneous HPLC analysis of catecholamines and indoleamines in mouse brain tissue following acetate extraction and treatment with ascorbate oxidase. M.G. Hadfield, C. Milio and N. Narisimhachari.

Die früher (M.G. Hadfield et al., J. Liquid Chromatogr. **8,** 2689 (1985)) entwickelte Methode zur Bestimmung von Monoaminen im Gehirn wurde verbessert, um weitere Gehirngebiete zu erfassen. Die Anwendung einer Essigsäure/Ascorbatoxidase-Extraktion (M. Frankfurt et al., Brain Res. **340,** 127 (1985)) verminderte die Front und gestattete bessere Erhaltung der Verbindungen. – *Arbeitsweise.* Das Gehirngewebe wurde mit Na-Acetatpuffer (pH 5,0) extrahiert, das Homogenat filtriert und zentrifugiert. Dem Überstand wurde Ascorbatoxidase zugegeben, und dann wurde an einer Supelcosil ODS 5 µ C-18-Säule chromatographiert. Die mobile Phase bestand aus einer in deionisiertem Wasser gelösten Mischung von 0,1 M Citronensäure, 0,06% Diethylamin, 0,05 mM EDTA, 200 mg/l 1-Heptansulfosäure und 4,5% CH_3CN bei pH 2,5. Die Trennung erfolgte isokratisch bei Raumtemperatur. – J. Liquid Chromatogr. **10,** 2439–2446 (1987). Neurochem. Res. Lab., Dept. Pathol., Commonwealth Univ., Richmond, VA (USA) J. Eliassaf

HPLC determination of monoamines in rat brain after enzymatic treatment with ascorbate oxidase and sulfatase. M.G. Hadfield and C. Milio.

Die gleichzeitige Analyse von Norepinephrine, Dopamine und Serotonin und deren Metaboliten, MHPG, DOPC und 5HIAA im Rattengehirn gelang mit der HPLC-Methode nach M.G. Hadfield et al. (J. Liquid Chromatogr. **8,** 184, 2689 (1985)). Eine scharfe Trennung wurde durch vorangehende Inkubation mit Sulfatase und Ascorbatoxidase erzielt. – J. Liquid Chromatogr. **10,** 2447–2452 (1987). Neurochem. Res. Lab., Dept. Pathol., Med. College, Commonwealth Univ., Richmond, VA (USA) J. Eliassaf

Combined cation exchange-alumina extraction of [^{3}H]noradrenline for the determination of [^{3}H]noradrenaline plasma kinetics in man. L.G. Howes and P.R. Rowe.

Verff. verwenden zur Adsorption von [^{3}H]-Noradrenalin (NA) aus Humanplasma das Kationen-Austauscher-Harz Biorad AG50W-X4, 200–400 mesh, welches nach Reinigung (Verfahren s. Original) in eine Plastiktüte gepackt und nochmals gewaschen wird (Einzelheiten s. Text). 4 ml Plasma werden, nach Zusatz von 4 mg Dihydrobenzylamin als interner Standard und 500 µl einer EDTA-/Na-metabisulfitlösung auf das Harz gegeben und gründlich gewaschen. Die Eluierung des NA und [^{3}H]NA erfolgt durch 260 µl 0,2 M Perchlorsäure; vom Eluat werden 50 µl in das HPLC-System injiziert. – Scand. J. Clin. Lab. Invest. **47,** 749–750 (1987). Dept. Med., Univ., Melbourne (AUS) K. Söllner

Chromatographic analysis and purification of multiply tritium-labelled eicosanoids. V.P. Shevchenko, I.Yu. Nagayev and N.F. Myasoyedov.

This is a comparative study of different chromatographic techniques (GLC, TLC, LC, HPLC) as applied to the analysis and preparative purification of tritium-labelled eicosanoids with a molar radioactivity of 1.8–8.8 TBq/mmol obtained by selective hydrogenation and by chemical or enzymic methods. We demonstrate the possibility of analyzing reaction mixtures and isolating individual multiply labelled eicosanoids with a chemical and radiochemical purity of 95–98%. Special features of HPLC for high molar radioactivity eicosanoids are considered. – J. Radioanal. Nucl. Chem., Art. **121,** 479–487 (1988). Inst. Mol. Genet., Acad. Sci., Moscow (SU)

Ligand exchange chromatography for analysis and preparative separation of tritium-labelled amino acids. Yu.A. Zolotarev, D.A. Zaitzev, V.I. Penkina, I.N. Dostavalov and N.F. Myasoyedov.

Racemic tritium-labelled amino acids were separated into optical isomers by chromatography on a chiral polyacrylamide sorbent filled with copper ions. The polyacrylamide sorbent is synthesized by Mannich's reaction through the action of formaldehyde and L-phenylalanine upon polyacrylamide Biogel P-4 in an alkali phosphate buffer. Tritium-labelled amino acids are eluted by a weak alkali solution of ammonium carbonate. Data are presented on the ligand exchange chromatography of amino acids depending on the degree to which the sorbent is filled with copper ions and on the eluent concentration. Conditions are suggested for the quantitative separation of amino acid racemates. Amino acids are isolated from the eluent on short columns filled with sulfonated cation exchanger in the H^+ form. HPLC on modified silica gel sorbents is also used for the analysis of tritium-labelled optically active amino acids. Amino acids are eluted by a weakly acidic water-methanol solution containing ammonium acetate. UV and scintillation flow type detectors are used. – J. Radioanal. Nucl. Chem., Art. **121,** 469–478 (1988). Inst. Mol. Genet., Acad. Sci., Moscow (SU)

Analysis of amino acids in brain and plasma samples by sensitive gas chromatography-mass spectrometry. A.K. Singh and M. Ashraf.

GS-MS-SIM provided a simple and sensitive method for analyzing amino acids in plasma and brain samples. Although the sensitivities of chemical ionization and electron-impact ionization were similar chemical ionization produced higher-mass ions, which might increase the selec-

tivity of the assay. Both chemical and electron-impact ionization distinguished the natural amino acids from the ^{15}N-labelled amino acids. The recovery of amino acids from plasma and brain samples was ca. 75%. The amino acid levels determined by GC/MS were comparable with the amino acid levels determined by HPLC or amino acid analyzer. – J. Chromatogr. **425,** 245–255 (1988). Dept. Veterin. Diagn. Invest., College Veterin. Med., Univ., St. Paul, MN (USA)

Rapid and efficient separation of PTH-amino acids employing supercritical CO_2 and an ion pairing agent. M. Ashraf-Khorassani, M.G. Fessahaie, L.T. Taylor, T.A. Berger and J.F. Deye.

Zur Sequenzanalyse von Proteinen nach Edmann ist die analytische Trennung von Phenylthiohydantoin-Aminosäuren (PTH-AA) wichtig. Bedeutsame Parameter sind: Raschheit der Analyse, Trennung auf der Basislinie, die Reproduzierbarkeit und die niedrige Nachweisgrenze. Zu diesem Zweck wird eine gepackte stationäre Cyanopropyl-Phase mit einem Gradienten von überkritischem CO_2 und Methanol/Tetramethylammoniumhydroxid eluiert. So wird beispielsweise in 18 min ein Gemisch von 24 Aminosäurederivaten aufgetrennt. Die Wahl der Analysenbedingungen wird diskutiert. – J. High Resolut. Chromatogr. **11,** 352–353 (1988). Virginia Polytechnic Inst., State Univ., Dept. Chem., Blacksburg, VA (USA) B.R. Glutz

High-performance liquid chromatographic determination of plasma histamine after pre-column derivatisation with o-phthaldialdehyde. R. Czerwonka, D. Tsikas and G. Brunner.

This method includes selective extraction of histamine from plasma, pre-column derivatisation in aqueous phase with o-phthaldialdehyde (OPA) and HPLC analysis. The fluorescence of the histamine-OPA-complex was monitored at wavelengths of 350 nm excitation and 450 nm emission, after isocratic eluation with a mixture of 0.2 M NaCl and methanol. The reproducibility of this method including extraction, derivatisation and detection of histamine was >95% in a range of 0.35–17.6 pmol. The HPLC precision was 99±1% at 4 pmol of histamine. The lower limit of detection was 88 fmol. A significantly increased concentration of plasma histamine was detected in patients (n=46) with various liver diseases (0.3–5.2 ng/ml). In comparison the plasma histamine levels of healthy blood donors were in the range of 0.0–0.4 ng/ml ($p < 0.01$). – Chromatographia **25,** 219–222 (1988). Dept. Gastroenterol. Hepatol., Coll. Med. Hannover, Oststadt Hosp., Hannover (D)

Liquid chromatographic determination of diastereomeric glutathione conjugates and further derivatives of α-bromoisovalerylurea in rat bile and urine by electrochemically generated bromine. J.M. Te Koppele, E.J. van der Mark and G.J. Mulder.

The separation characteristics of a bromoisovalerylurea metabolites and the corresponding cysteine conjugates were investigated. All thioether metabolites could be separated in one run, optimal separation of the diastereomers required different mobile phases for the glutathione conjugates (in bile) and the mercapturates (in urine). The glutathione conjugates were analysed with the ion-pairing agent sodium decanesulphonate in the mobile phase, but the mercapturates were analysed without an ion-pair-forming agent. For detection, on-line generation of a constant bromine level (100%) was used. This technique could be used in continuous automated operation and required little clean-up of the sample. The diastereomeric glutathione conjugated and mercapturates were quantified in rat bile and urine samples, respectively, by direct injection of the (centrifuged and diluted) samples on the column. The limit of determination of the respective metabolites was 9 and 2.6 ng in bile and urine, respectively. – J. Chromatogr. **427,** 67–77 (1988). Div. Toxicol., Center Bio Pharm. Sci., Univ., Leiden (NL)

Gradient reversed-phase liquid chromatography of proteins on very short columns. Influence of particle size on peak dispersion. M. Verzele, Y.-B. Yang, Ch. Dewaele and V. Berry.

This paper is an experimental contribution to the behavior of proteins in gradient RP-LC. Very short columns and the smallest available particle were used (1 cm column length and 1-, 2-, 3-, 5-, and 10-μm RO-Sil-C18-D-particles). Phenones (small molecules) and proteins (large molecules) were compared at different flow rates and different gradients. The decrease in peak dispersion with smaller particle size is more pronounced for proteins than for phenones. Very small particles (3, 2, over 1 μm) with a slow gradient but with high flow rate on a short column appear to be of interest for protein chromatography. – Anal. Chem. **60,** 1329–1332 (1988). Lab. Org. Chem., State Univ., Gent (B)

Evaluation of an immunoturbidimetric microalbuminuria assay. L. Paloheimo, M. Pajari-Backas, E. Pitkänen, L. Melamies and R.R. Rissanen.

Eine zentrifugierte Harnprobe wird mit Albustix versetzt und bei positivem Ausfall der Reaktion mit NaCl-Lösung auf einen Albumin(A)-Gehalt zwischen 5–100 mg/l verdünnt. Nach Zusatz von Antiserum (porcine anti-human-albumin serum s. Original) wird die entstandene Trübung bei 30°C mit 340 nm Wellenlänge gemessen. Die Nachweisgrenze liegt bei 5 mg A/l, die Rückgewinnungsrate zwischen 98,6 und 106,6%. Die Resultate zeigen gute Übereinstimmung mit den mittels Radial-Immunodiffusion bzw. Radioimmuntest erhaltenen Werten. – J. Clin. Chem. Clin. Biochem. **25,** 889–892 (1987). United Labs. Ltd., Helsinki (SF) K. Söllner

Determination of nonenzymatically glycated albumin and IgG by affinity chromatography and colorimetry. P.S.M. Caines, R.J. Thibert and T.F. Draisey.

A colorimetric procedure was developed for the sequential determination of nonenzymatically glycated IgG (GIgG) and albumin (GA). IgG and then albumin were separated from serum or plasma using a single DEAE Cibacron blue F3GA Affi-Gel column. The stable ketoamine linkages present in GIgG and GA reduced a tetrazolium salt to its colored formazan. The method was linear over the range 2.0–25.0% GIgG and 4.0–18.0% GA. For both GIgG and GA, the within- and between-run CV's were <4.5 and 8.5%, respectively, and recoveries were quantitative. The labile aldimine fraction, free glucose, and ascorbate did not affect the results. Nondiabetic reference intervals were 13.2–16.8% GIgG and 6.2–9.7% GA. Nondiabetic and diabetic populations can be clearly discriminated ($P < 0.005$). When compared with the thiobarbituric acid assay, the correlation coefficients for GIgG and GA were $r = 0.98$ and 0.97, respectively. In a mixed group of diabetic and nondiabetic subjects, glycated hemoglobin levels were compared with those of GIgG and GA, and $r = 0.68$ and 0.35, respectively. – Microchem. J. **37,** 70–76 (1988). Dept. Chem. Biochem., Univ., Windsor, Ont. (CDN)

Gradient gel electrophoresis immunoblot analysis (GGEI): A sensitive method for apolipoprotein profile determinations. M. Lefevre, J.C. Goudey-Lefevre and P.S. Roheim.

Das im Titel genannte Verfahren erfordert die folgenden Arbeitsgänge: 1.) Elektrophorese von 1–2 μl Plasma auf nicht denaturiertem Acrylamid-Gradient-Gel. 2.) elektrophoretische Überführung der Lipoproteine (Lp) auf modifizierte Nylon-Membrane. 3.) Fixierung der Lp mit Glutaraldehyd. 4.) Immunolokalisation der Apo-Lp mit iodierten monospezifischen Antikörpern. 5.) Autoradiographie und Densitometrie. 6.) Quantitation und Ermittlung der Apo-Lp im Gewebe. Die Arbeit enthält mehrere Elektropherogramme und Diagramme. – J. Lipid Res. **28,** 1495–1507 (1987). Dept. Physiol., State Univ., Med. Center, New Orleans, LA (USA) K. Söllner

Standardisation of an enzymometric assay for apolipoprotein A-1 by using mixtures of monoclonal antibodies. Ch. Betard, N. Vu-Dac, H. Mezdour, A.Ch. Nestruck, A. Leroy and J.-Ch. Fruchart.

Unter Bezugnahme auf 28 Veröffentlichungen geben Verff. Anweisungen zur Isolierung von Liprotein aus Plasmaproben, Präparation monoclonaler Antikörper für Apolipoprotein A-1, deren Charakterisierung und Selektion sowie zur Durchführung immunoenzymometrischer Tests. Die Arbeit enthält mehrere Diagramme, aus denen die Auswertung für die Menge des A-1 in Plasma zu entnehmen ist. – J. Clin. Chem. Clin. Biochem. **25,** 893–899 (1987). Serv. Recher. Lipoproteins, Inst. Pasteur, Lille (F); Inst. Recher. Clin., Montreal Qué (CDN) K. Söllner

Separation and isolation of human apolipoproteins C-II, C-III_0, C-III_1 and C-III_2 by chromatofocusing on the fast protein liquid chromatography system. M.W. Huff and W.L.P. Strong.

Chromatofokussierung, welche Proteine auf der Grundlage unterschiedlicher isoelektrischer Punkte trennt, wird zur schnellen Protein-Flüssigchromatographie (FPLC; Pharmacia, Montreal, Quebec, CDN), mit der man die C Apolipoproteine von humanen Lipoproteinen sehr geringer Dichte (VLDL) abtrennen kann, eingesetzt. Mit Hilfe einer Mono P Säule (Pharmacia) wird ein pH-Gradient zwischen pH 6,2 und pH 4,0 unter Verwendung verschiedener Puffer in 6 M $(H_2N)_2CO$ bei einer Fließgeschwindigkeit von 0,5 ml/min erzeugt. Typische Analysengänge nehmen ≈45 min in Anspruch. Fällung von VLDL mit $(H_3C)_2CO$ vor der Delipidierung beseitigt beide Apolipoproteine E und B. Unter Verwendung eines Startpuffers aus 25 mM Histidin (pH 6,2) und einer 1:30 Verdünnung des Polypufferaustauschers (Laufmittelpuffer; Polybuffer 74, Pharmacia) werden apoC-II, C-III_0, C-III_1 und C-III_2 in ≈60 min Laufzeit gut aufgetrennt. Für die chromatographierten Proteine resultieren Wiederfindungsraten >90%. Diese Technik ist gegenüber gewöhnlichen Vorgehensweisen aufgrund ihrer Zeitersparnis und hohen Auflösungsvermögens überlegen. – J. Lipid Res. **28,** 1118–1123 (1987). Dept. Med., Univ. Hosp., Univ. West. Ontario, London, Ontario (CDN)
F.T. Bartsch

Discontinuous reversed-phase high-performance liquid chromatography increases load capacity of analytical columns. Separation of ribosomal proteins from the archaebacterium *Sulfolobus acidocaldarius*. J.R. Grün, B. Kossmann and R. Reinhard.

Since ribosomes are a fundamental feature component of all organisms, they present a good model for studying evolutionary diversity. We investigated the ribosomal proteins of the archaebacterium *Sulfolobus acidocaldarius,* which contains slightly more ribosomal proteins than *Escherichia coli.* While the ribosomal proteins of most organisms contain a high proportion of lysine and arginine residues, these basic amino acids are particularly prevalent in the thermoacidophile organism *Sulfolobus,* a possible reason for poor separation of total ribosomal proteins of *S. acidocaldarius* by single column HPLC. To solve this complex separation problem, we developed the discontinuous reversed-phase HPLC (Disc RPC) method. Discontinuous chromatography combines at least two different stationary phases in a sequence of increasing retention times for the elution of proteins. Unlike other multi-column techniques, all of the injected sample passes through this discontinuous stationary phase, which is used in place of single columns, thus permitting separations to be carried out without the need of any changes in the HPLC system. – Chromatographia **25,** 189–198 (1988). Max-Planck-Inst. Mol. Genet., Abt. Wittmann, Berlin (D)

Separation of 50S ribosomal proteins from *Sulfolobus acidocaldarius* by discontinuous reversed-phase chromatography. B. Kossmann, J.R. Grün and R. Reinhardt.

The total protein of the 50S ribosomal subunit (TP50) from the archaebacterium *Sulfolobus acidocaldarius* was prefractionated by discontinuous reversed-phase HPLC with several column combinations. The purity of the eluted fractions was tested by SDS-PAGE or two-dimensional PAGE. With regard to the load capacity and selectivity, best results were obtained with a semi-preparative three-column combination (total length 67.5 cm). Twelve A_{230} units of TP50 were separated into 35 fractions, 19 of which contained nearly pure proteins. When the sample load was increased to 176 A_{230} units (109 mg), 13 of 28 collected fractions still contained nearly pure proteins. The selectivity of a semi-preparative short-column combination (total length 12 cm) was similar to that of the semi-preparative column combination, and separation time could be reduced to one third of that required for the longer column combination. The load capacity of the short-column combination was lower than that of the semi-preparative column combination. – Chromatographia **25,** 215–218 (1988). Max-Planck-Inst. Mol. Genet., Abt. Wittmann, Berlin (D)

Thermospray-mass spectrometric analysis of underivatized monohydroxy fatty acids: Application to stimulated platelets. M. Guichardant, M. Lagarde, M. Lesieur and F. De Maack.

Monohydroxylated fatty acids prepared from polyunsaturated fatty acids of nutritional value were analysed by thermospray-mass spectrometry without prior chemical derivatization. Positive and negative ionization modes were compared. The highest sensitivity was observed with the negative ionization mode with detection limits of 10 pmol based on the 12-hydroxy derivative of eicosatrienoic acid (12-OH-8,10,14-20:3). This is comparable to that obtained by HPLC with UV detection at 234 nm. Selected ion monitoring based on the fragment $[M\text{-}H]^-$ allowed a variety of standard monohydroxy fatty acids to be detected. This approach makes possible the analysis of various derivatives generated by thrombin-stimulated platelets (10^9 cells) pre-enriched with minor polyunsaturated fatty acids, even when these derivatives co-elute from the column (e.g., 12-HETE and 14-OH-22:6). – J. Chromatogr. **425,** 25–34 (1988). Inst. Pasteur, INSERM U63, Lab. Hémobiol., Fac. Alexis Carrel, Lyon (F)

The development of more sensitive solvent systems for separation of cholesterol and low-molecular-weight cholesteryl esters by thin-layer chromatography. G.W.C. Hung and A.Z. Harris.

Four solvent systems have been developed for more effective one-stage separation of cholesterol and its low-molecular-weight esters from their mixtures by one-dimensional TLC. These four solvent systems have been tested for separation of cholesterol, cholesteryl formate, cholesteryl acetate, and cholesteryl propionate from their mixtures and compared to the results of separation by two-stage solvent system, one-dimensional TLC techniques reported in the literature. – Microchem. J. **37,** 174–180 (1988). Dept. Chem., Univ. Motevallo, AL (USA)

Determination of coenzyme Q_{10}, α-tocopherol and cholesterol in biological samples by coupled-column liquid chromatography with coulometric and ultraviolet detection. P.O. Edlund.

Coenzyme (Co) Q_{10}, Co $Q_{10}H_2$, α-tocopherol and cholesterol were dissociated from lipoproteins in plasma by treatment with 1-propanol. The supernatant obtained was injected directly for determination of Co Q_{10} and Co $Q_{10}H_2$. Precolumn reduction with borohydride was used for determination of total Co Q_{10} simultaneously with α-tocopherol and cholesterol. Total Co Q_{10} in freeze-dried myocardial biopsies was determined after extraction with 1-propanol and oxidation of Co $Q_{10}H_2$ with ferric chloride. The chromatographic system comprised two reversed-phase columns and a three-electrode coulometric detector and a UV detector coupled in series. A pre-fractionation on the first column protected the coulometric detector from contamination and reduced the time for analysis by eliminating strongly retained solutes. The coulometric electrodes were operated in the oxidation-reduction-oxidation mode, and the last electrode was used for detection of α-tocopherol, Co Q_{10} and Co $Q_{10}H_2$, while cholesterol was detected by UV at 215 nm. The fast isolation procedure made it possible to determine the reduced and oxidized forms of Co Q_{10} in plasma. Quantitative recoveries were obtained for all the analytes studied and normal levels were determined with a coefficient of variation of 2–3%. – J. Chromatogr. **425,** 87–97 (1988). Bioanal. Sect., Res. Devel., ACO Läkemedel AB, Solna (S)

Determination of cortisol in human plasma by capillary gas chromatography-mass spectrometry using [$^2H_5$]cortisol as an internal standard. N. Hirota, T. Furuta and Y. Kasuya.

A capillary GC-MS method for the determination of cortisol in human plasma using cortisol M+5 as an internal standard is described. For calculation of plasma cortisol, peak areas were measured by selected-ion monitoring of the characteristic fragment ions of the dimethoxime-tri(trimethylsilyl) derivatives of cortisol and cortisol M+5 (m/z 605 and 610, respectively). The inter- and intra-assay coefficients of variation for plasma sample were 3.07 and 1.77%, respectively. The method needed no complex corrections for contributions and provides a sensitive and reliable technique with good accuracy, precision and reproducibility. – J. Chromatogr. **425,** 237–243 (1988). Tokyo College Pharm., Tokyo (J)

Selective determination of urinary free cortisol by liquid chromatography after solid-state extraction. E.P. Diamandis and M. D'Costa.

We have developed a selective and precise method with an improved and efficient sample clean-up using C_{18} Sep-Pak cartridges. The urine sample (2 ml), with 11-deoxycortisol as internal standard, is applied to the Sep-Pak, which is then sequentially washed with acetone/water (1:4), water and hexane. Cortisol is eluted with diethyl ether, evaporated to dryness and redissolved in 2 ml of water. The wash cycle is repeated once using the same Sep-Pak cartridge. This double extraction greatly

improves sample clean-up and allows modification of the mobile phase (tetrahydrofuran/methanol/water) so that cortisol is rapidly eluted as a single well resolved peak at 13 min. Chromatography is performed isocratically on a reversed-phase column with detection at 254 nm. Detection limits for urinary free cortisol by this procedure were two or three times lower than those obtained with two commercial radioimmunoassay kits. – J. Chromatogr. **426,** 25–32 (1988). Dept. Clin. Biochem., Mount Sinai Hosp., Univ. Toronto, Ontario (CDN)

Simultaneous determination of estrogen and progesterone receptors using human uterus as a standard. N.Y. Zachariah, J. Osborne and Z.H. Chakmakjian.

Bei der Frage der Korrelation zwischen Anwesenheit von Östrogen- und Progesteron-Receptoren in Brusttumorgeschwulsten und der Ansprechbarkeit endokriner Therapie steht die quantitative Erfassung dieser Receptoren im Mittelpunkt. Im Labor der Verff. wurde eine gegenüber den bekannten umständlichen Verfahren schnellere und einfachere Methode ausgearbeitet. Dabei wird menschlicher Uterusextrakt mit bekannter Receptorkonzentration als Standard benutzt. Einzelheiten des sehr weit im klinisch-chemischen Bereich angesiedelten Verfahrens s. Original. – Anal. Lett. **21,** 367–380 (1988). Endocrin. Lab. Dep. Pathol., Baylor Univ. Med. Center, Dallas, TX (USA) W. Czysz

Quantitative analysis of 6-keto-prostaglandin $F_1\alpha$ using immunoaffinity purification and gas chromatography-mass spectrometry. J.J. Vrbanac, T.D. Eller and D.R. Knapp.

This paper describes an immunoaffinity purification technique for 6-keto-prostaglandin $F_1\alpha$ ($6KPGF_1\alpha$) prior to quantitative by HRGC-NICIMS. Polyclonal antibodies to $6KPGF_1\alpha$ were partially purified using *Staphylococcus aureus* Protein A immobilized on Sepharose CL-4B. This partially purified fraction was covalently bound to silica gel using N-hydroxysuccinimidyl-functionalized silica. Columns constructed using this gel quantitatively bound $6KPGF_1\alpha$ which could be eluted quantitatively with acetonitrile/water (19:1). Binding capacity was reconstituted by washing with 0.01 M phosphate buffer (pH 7.4). Human urinary and canine plasma $6KPGF_1\alpha$ was sufficiently purified using these columns that HRGC-NICIMS analysis of the methoxime-pentafluorobenzyl-tris-trimethylsilyl derivatives was interference-free. – J. Chromatogr. **425,** 1–9 (1988). Dept. Cell. Molecular Pharmacol. Exp. Therapeutics, Med. Univ., Charleston, SC (USA)

Determination of insulin in human saliva using a more sensitive sandwich enzyme immunoassay. Ke-he Ruan, Dan-ru Ke, Xiu-wang Huang, Daren Ni, Shi-zhang Pan, Rui-hong Yao, Hao-xue Lin and Zhi-chun Xie.

Zur Bestimmung von Insulin mit einem empfindlichen Enzymimmunoassay-Verfahren (EIA) in Sandwichtechnik inkubiert man eine mit Anti-Insulin-Antikörper-IgG überzogene Polystyrolkugel (∅ 3,2 mm) zusammen mit Insulin oder der Realprobe in einem Gesamtvolumen von 50 µl in einem kleinen Glasröhrchen (3,5 × 20 mm) und anschließend mit affinitätsgereinigtem (Anti-Insulin) Fab'-Peroxidase-Konjugat (ebenfalls 50 µl-Volumen) in einem gleichen Glasröhrchen. Die an die Kugel gebundene Peroxidase-Aktivität wird fluorimetrisch bestimmt. Der gefundene Wert entspricht der Insulinkonzentration; Nachweisgrenze 0,3 nU (12,5 fg) pro Röhrchen. Dieses Verfahren wird auf die Insulinbestimmung in Speichel übertragen. Hier wurde als normaler Insulinpegel eine Konzentration von 2,8 µU/ml (Standardabweichung 2,47) bestimmt. – Anal. Lett. **21,** 381–394 (1988). Biochem. Central Lab., Fujian Med. Coll., Fuzhou, Fujian (RC) W. Czysz

Size-exclusion and high-performance liquid chromatography separation of peptides from peptic haemoglobin hydrolysate obtained by ultrafiltration. J.M. Piot, D. Guillochon and D. Thomas.

Gel filtration (size-exclusion) and HPLC have been used to separate peptic peptides from haemoglobin hydrolysate. Elution profiles on Sephadex G-25 displayed nine fractions with molecular weights lower than 6500 daltons. Each fraction was analysed for total amino acid content and showed less than 1% free amino acids. Reversed-phase HPLC, using ammonium acetate buffer and acetonitrile as solvent, was applied to each fraction in order to obtain pure peptide peaks. The importance of acquiring a better knowledge of such an hydrolysate is discussed. Various potential applications of this type of hydrolysate, some of them already being undertaken, are envisaged. – Chromatographia **25,** 307–312 (1988). Lab. Technol. Subst. Natur., I.U.T. "A" de Lille I, Villeneuve d'Ascq (F)

Application of reversed-phase and ion-pair HPLC for investigation of nucleotide metabolism in erythrocytes, reticulocytes and tumour cells. A. Werner, W. Siems, G. Gerber, H. Schmidt, S. Gruner and H. Becker.

The determination of nucleotides, nucleosides, and nucleobases was carried out in cells of different metabolic complexity: in mature and immature red blood cells, in Ehrlich ascites tumour cells from different proliferation stages, and in other tumour cells. The maturation of reticulocytes to erythrocytes is accompanied by loss of organelles and energy-requiring processes as well as the switch from aerobic to anaerobic ATP production. The profile of purine nucleotides, nucleosides, bases, and pyridine dinucleotides, by reversed-phase HPLC, shows large concentration changes during the maturation of red blood cells. The concentrations of purine mono and triphosphates are two to four times greater in reticulocytes in comparison with erythrocytes; the difference in the concentrations of nucleosides and nucleobases between reticulocytes and erythrocytes is even greater. – Chromatographia **25,** 237–240 (1988). Inst. Biochem., Med. Fac. (Charité), Humboldt Univ., Berlin (DDR)

Diagnostic and therapy-control of inborn metabolic disorders by high-performance liquid chromatography: 2,8-dihydroxyadeninuria, xanthinuria. A. Hesse, A. Thon, A. Classen and H. Birwé.

2,8-Dihydroxyadeninuria and xanthinuria are inborn enzyme disorders which must be under lifelong therapeutic control. Quantitative determination of 2,8-DHA and xanthine using reversed phase HPLC was performed. A new application for the separation of purines shortens analysis-time. Examples of the determination of 2,8-DHA and xanthine before and under certain therapies are shown. Long term monitoring of the patients offers the possibility of a reliable prophylaxis against stone recurrence. – Chromatographia **25,** 205–209 (1988). Experim. Urol., Urol. Univ. Klinik, Bonn (D)

Fluorimetric determination of aldose reductase in small tissue samples. S.E. Brolin, P.-O. Berggren and P. Naeser.

Das beschriebene Verfahren beruht auf dem durch das Enzym Aldose-Reduktase katalysierten Verbrauch von NADPH unter Verwendung von Glucose als Substrat. Es entsteht Sorbitol + $NADP^+$, gemessen wird die Abnahme der ursprünglichen NADPH-Fluorescenz, und zwar als Differenz von zwei Ansätzen, einer mit und einer ohne Glucose. Die Methode ist eine Modifikation des Verfahrens mit Messung der Absorption bei 340 nm; das fluorimetrische Verfahren ist 10mal empfindlicher. Man arbeitet mit Gewebeproben von 0,1–2 mg. – Anal. Chim. Acta **206,** 357–361 (1988). Dep. Med. Cell Biol., Biomed. Center, Univ., Uppsala (S) W. Czysz

Methods for the determination of the interferon-induced enzyme 2',5'-oligodenylate synthetase in mononuclear blood cells. G. Bruchelt, J. Beck, K. Schilbach-Stückle, E. Koscielniak, J. Treuner and D. Niethammer.

Unter Auswertung von 34 einschlägigen Veröffentlichungen besprechen Verff. die bisher angewandten vier Verfahren zur Bestimmung des o.g. Enzyms in der monomolekularen Zellfraktion des peripheren Blutes. Die Arbeit enthält eine schematische Darstellung der einzelnen Arbeitsgänge und deren kritische Beurteilung (Wertetabellen und Diagramme im Original). – J. Clin. Chem. Clin. Biochem. **25,** 879–888 (1987). Abt. Hämatol. Onkol., Univ.kinderklinik, Tübingen (D) K. Söllner

Determination of activity and amount of silica-immobilized penicillinase. M.C. Gosnell and H.A. Mottola.

Several aspects of the determination of immobilized penicillinase (EC 3.5.2.6) on borosilicate glass and on controlled-pore glass by attachment/detachment of a dye [Brilliant blue G (C.I. 42655)] are described. Under optimum conditions for protein determination enzyme activity is lost. A modified method using conditions that do not affect the catalytic activity of the enzyme when borosilicate glass is used as inert matrix is also presented. This modified method, however, fails to preserve activity if the inert matrix is controlled-pore glass. The sensitivity of the modified method is lower than that of the unmodified one because of a lower

stoichiometric ratio for the dye/protein interaction. The ratio is surface and pH dependent. The correlation between amount of protein immobilized and enzyme activity is examined. – Microchem. J. **37,** 149–154 (1988). Dept. Chem., State Univ., Stillwater, OK (USA)

Improved method for the determination of phospholipase A_2 catalytic activity concentration in human serum and ascites. H.R. Schädlich, M. Büchler and H.G. Beger.

Eine verbesserte radiochemische Methode zur selektiven Bestimmung der Aktivität von Phospholipase A_2 in Humanserum und Bauchwassersuchtproben unter Verwendung gewöhnlich erhältlicher Reagentien läßt sich auf große Probenmengen anwenden. Als Substrat wird 1,2-Dipalmitoyl-sn-glycero(3)phosphorylcholin und Phosphatidylcholin mit in Position 2 Tritium-markierter Palmitinsäure (1-Palmitoyl-2-[9,10-^{3}H]palmitoyl-sn-glycero(3)phosphorylcholin) eingesetzt. Die Radioaktivität der freigesetzten Fettsäuren wird nach Anwendung eines umfangreichen Extraktionsverfahrens in einem Flüssigszintillationsspektrometer registriert. Eine vorläufige Bezugsreihe von Humanserumproben wird bis zu 1,0 U/l eingerichtet. Im Serum von Patienten mit akuter Bauchspeicheldrüsenentzündung werden Aktivitäten bis zu 20 U/l gefunden. Die Korrelation der Aktivität von Phospholipase A_2 mit jener anderer Enzyme (pankreatische Isoamylase und immunoreaktive Lipase) und mit der Schwere und den Komplikationen akuter Pankreatitis wird untersucht. Eine mögliche Beziehung zwischen Phospholipase A_2-Aktivitäten und Lungenkomplikationen wird erörtert. – J. Clin. Chem. Clin. Biochem. **25,** 505–509 (1987). Abt. Allg. Chir., Univ., Ulm (D)
F.T. Bartsch

A fluorometric coupled enzymatic method for the determination of succinic semialdehyde dehydrogenase in lymphocytes and platelets. P.S.M. Caines, R.J. Thibert and T.F. Draisey.

A fluorometric, coupled enzymatic method for the determination of succinic semialdehyde dehydrogenase (SSA-DH) in lymphocytes and platelets is described. The limits of linearity of the method are 0.52–15.0 pmol/min/mg protein. Within- and between-run CVs were both <6.5% and recoveries were quantitative. Mean SSA-DH levels in lymphocytes and platelets of "normal" subjects were 7.5 ± 1.4 pmol/min/mg protein and 7.5 ± 1.9 pmol/min/mg protein, respectively. – Microchem. J. **37,** 216–220 (1988). Dept. Chem. Biochem., Univ., Windsor, Ont. (CDN)

Determination of drugs in human serum by liquid chromatography/atmospheric pressure ionization mass spectrometry. M. Sakairi and H. Kambara.

A rapid and highly sensitive method to determine drugs in human serum by liquid chromatography/atmospheric pressure ionization mass spectrometry (LC/API-MS) is reported. This method has wide applicability and high sensitivity, with detection limits ranging from ng/ml to µg/ml. Experimental results demonstrate that the LC/API-MS is very promising for determination of drugs in human serum. – Anal. Sci. **4,** 199–201 (1988). Adv. Res. Lab., Hitachi Ltd., Tokyo (J)

Thin layer chromatographic analysis of basic and quaternary drugs extracted as bis(2-ethylexyl)phosphate ion-pairs. I. Ojanperä and E. Vuori.

Die DC-Bestimmung von Arzneimitteln aus den Gruppen quaternäre Ammoniumbasen, sekundäre und tertiäre Amine (Tabelle mit 19 Substanzen s. Text) in Harn erfolgt auf 10 × 20 cm-Platten Silica Gel 60 F 254 (No. 5554) oder R-P-18 F_{254}s (No. 15423) von Merck. 5 ml Harn werden nach Ansäuern mit HCl auf pH 2–3 10 min lang mit 10 ml Ether geschüttelt und zentrifugiert. Die wäßrige Phase wird mit NaOH auf pH 7 eingestellt, mit 2 ml 0,1 M Sörensen-Phosphatpuffer pH 7,0 versetzt und 15 min lang mit 10 ml Dichlormethan, welches 0,01 M Bis(2-ethylhexyl)phosphorsäure enthält, geschüttelt. Nach Zentrifugieren wird die CH_2Cl_2-Phase evaporiert und der Rückstand mit 100 µl einer Mischung von 70% CH_3OH und 30% 1 M HCl geschüttelt und zentrifugiert. 5 µl des Überstandes werden auf die DC-Platten aufgetragen. Die Entwicklung erfolgt mit 8 DC-Fließmittelsystemen (Zusammensetzung und R_f-Werte im Original). – J. Liquid Chromatogr. **10,** 3595–3604 (1987). Dept. Forensic Med., Univ., Helsinki (SF)
K. Söllner

Sensitive high-performance liquid chromatographic determination of pseudoephedrine in plasma and urine. E. Brendel, I. Meineke, E.-M. Henne, M. Zschunke and D. de Mey.

Ein HPLC-Verfahren zur Bestimmung von Pseudoephedrin, einem Erkältungsmittel, in Plasma wird beschrieben, mit dem die quantitative Bestimmung bis hinunter zu 10 ng/ml in einer 1 ml-Plasmaprobe möglich ist. Dazu werden die Plasmaproben mit einer salzsauren Lösung von α-(Methylaminomethyl)benzylalkohol versetzt, Ammoniak zugegeben und über eine BondElut-Extraktionssäule gegeben. Nach Waschen wird mit 0,1 M methanolischer HCl eluiert. Der Rückstand des eingedampften Eluats wird in Wasser aufgenommen und auf einer µBondapak C_{18}-Säule mit einer mobilen Phase aus 0,03 M Natriumheptansulfonat (pH 3)/Acetonitril (77:23) analysiert. Der Nachweis kann bei 220 nm im UV durchgeführt werden. Eichkurven sind im Bereich von 10–500 ng/ml linear. Die Wiederfindungsraten liegen bei 85–88% für Pseudoephedrin und bei 74% für den internen Standard. Die Nachweisgrenze wird mit 5 ng/ml (S/N = 3) angegeben. – J. Chromatogr. **426,** 406–411 (1988). SK&F Inst. Appl. Clin. Pharmacol., Smith Kline Dauelsberg GmbH, Göttingen (D) R.H.S.

Complete separation of urinary metabolites of paracetamol and substituted paracetamols by reversed-phase ion-pair high-performance liquid chromatography. M.I. Aguilar, S.J. Hart and I.C. Calder.

A reversed-phase high-performance liquid chromatographic procedure has been developed for the separation of thirteen urinary metabolites of the analgesic drug paracetamol. The method involved the use of radially compressed columns packed with octadecylsilica with a particle diameter of 5 µm. Metabolites were chromatographed by linear gradient elution using an ion-pair solvent system composed of tetrabutylammonium hydroxide and Tris buffered to pH 5.0 with phosphoric acid, and acetonitrile as the organic solvent. Analyses can be performed at the rate of three per hour. This method enables the direct identification of sulphate and glucuronide conjugates of 3-thiomethylparacetamol and 3-thiomethylparacetamol sulphoxide which have only previously been detected following enzyme hydrolysis of urine samples. – J. Chromatogr. **426,** 315–333 (1988). Dept. Organ. Chem., Univ. Melbourne, Parkville, Victoria (AUS)

Enantiomeric analysis of new anti-inflammatory agent in rat plasma using a chiral β-cyclodextrin stationary phase. A.M. Krustulovic, J.M. Gianviti, J.T. Burke and B. Mompon.

Zur Enantiomeren-Bestimmung des neuen entzündungshemmenden Agens, der trans-6,6a,7,10,10a,11-Hexahydro-8,9-dimethyl-11-oxodibenz[b,e]oxepin-3-essigsäure (I) in Rattenplasma wird ein HPLC-Verfahren unter Verwendung einer chiralen β-Cyclodextrinsäule vorgeschlagen. Dazu wird die mit 1 M Phosphatpuffer (pH 2,5) angesäuerte Plasmaprobe mit Dichlormethan extrahiert. Der Rückstand der eingedampften organischen Phase wird in mobiler Phase aufgenommen und auf einer Cyclobond I-Säule mit 0,05 M KH_2PO_4 (pH 5,3)/Methanol (35:65) chromatographiert. Der Nachweis wird bei 254 nm im UV ausgeführt. Beide Enantiomeren werden mit einer Extraktionsausbeute von mehr als 90% wiedergefunden. Im Konzentrationsbereich von 0,5–5 µg/ml erhält man eine lineare Eichkurve, die Nachweisgrenze wird mit 0,085 µg/ml angegeben. Durch Einsatz eines geeigneten internen Standards könnte die Genauigkeit des Verfahrens verbessert werden. – J. Chromatogr. **426,** 417–424 (1988). L.E.R.S.-Synthelabo, Paris (F)
R.H.S.

Determination of verapamil and norverapamil in human biological material. Investigation of plasma concentrations after oral administration of two different verapamil formulations. C. Horne, H. Spahn, E. Mutschler and H. Knauf.

Es wird eine Methode zur simultanen Bestimmung von Verapamil, das weltweit zur Therapie kardiovaskulärer Erkrankungen eingesetzt wird, und seinem Hauptmetaboliten Norverapamil in Humanplasma beschrieben. Nach alkalischer Extraktion mit n-Heptan erfolgt die Abtrennung von Begleitsubstanzen durch Ionenpaarchromatographie (HPLC) und anschließende Messung der Eigenfluorescenz beider Substanzen (λ_{ex} = 278 nm, λ_{em} = 320 nm). Die Empfindlichkeit des Verfahrens (Nachweisgrenze <1 ng/ml) ist ausreichend für pharmakokinetische Untersuchungen nach Gabe therapeutischer Dosen. Die Anwend-

barkeit der Methode wird in einer klinischen Studie getestet. Es werden Plasmakonzentrationen zweier Verapamil-Zubereitungen zur oralen Applikation gemessen. In die Untersuchung wird auch der wirksame Hauptmetabolit Norverapamil miteinbezogen. – Arzneim.-Forsch. **37(II),** 956–959 (1987). Pharmakol. Inst. Naturwiss., Univ., Frankfurt/M. (D) F.T. Bartsch

Measurement of alphaprodine by selected-ion monitoring. B.R. Kuhnert, W.T. Brashear and C.D. Syracuse.

Ein empfindliches EI-MS-Verfahren mit SIM wird zur Analyse des synthetischen Narcotikums Alphaprodin (Nisentil) vorgeschlagen. Dazu werden die Blutproben nach bekannten Verfahren nach Alkalisierung mit Methyl-tert.-butylether extrahiert, in HCl rückextrahiert und dann nach erneuter Alkalisierung in Methylenchlorid extrahiert. Diese Phase kann unter Verwendung von 2(Ethyl-sec-butylamino)-N-(2,6-dimethylphenyl)acetamid als internem Standard auf einer Säule mit 3% SE-30/OV-17 (6:1) auf 80–100 mesh Supelcoport mit einem Temperaturprogramm von 210–250°C chromatographiert werden. Der Nachweis bei 70 eV wird bei m/z 712 durchgeführt. Andere Narcotika stören die Bestimmung nicht. Die Empfindlichkeit des Verfahrens reicht bis hinunter zu 2 ng/ml, bis zu 200 ng/ml hinauf sind die Eichkurven linear. Durchschnittliche Wiederfindungsraten von 84% werden erzielt. – J. Chromatogr. **426,** 392–398 (1988). Dept. Obstretic. Metropol. General Hosp., Case Western Res. Univ., Cleveland, OH (USA) R.H.S.

Determination of methyldopa in plasma using high-performance liquid chromatography with electrochemical detection. C. Dilger, Z. Salama and H. Jaeger.

Die bisherigen Methoden zum Nachweis von Methyldopa in biologischen Matrices sind entweder zu mühsam und zu zeitaufwendig in der Probenvorbereitung oder zu wenig empfindlich bzw. nicht spezifisch genug in der Messung. Damit ist deren Einsatz bei Pharmakokinetik- bzw. Bioverfügbarkeits-/Bioäquivalenzstudien eingeschränkt. Die hier vorgestellte HPLC-Methode besteht aus einem kurzen Aufarbeitungsschritt mit anschließender direkter Aufgabe auf eine ODS-Säule und elektrochemischer Detektion. Zur Aufbereitung wird heparinisiertes Blut mit Perchlorsäure versetzt, durchmischt, kurz gefroren und anschließend zentrifugiert. Ein Aliquot des Überstandes wird direkt injiziert. Die Nachweisgrenze der Methode liegt bei 50 ng/ml. Die Eichkurve verläuft linear im Bereich von 50–200 ng/ml. – Arzneim.-Forsch./Drug Res. **37(II),** 1399–1401 (1987). LAB GmbH & Co., Neu-Ulm (D) M.J. Rittich

Determination of chlormezanone in human plasma after administration of chlormezanone formulations. S.L. Ali and H. Blume.

Zur HPLC-Bestimmung von Chlormezanon in Humanplasma wird 1 ml von diesem, nach Zusatz von 100 mg Ammoniumsulfat mit 2 ml Ethylacetat (E) 2 min lang geschüttelt, dann 3 min lang ultrabeschallt und 10 min lang mit 6000 U/min zentrifugiert. 1,00 ml der E-Phase wird zur Trockne evaporiert und der Rückstand in 0,5 ml der mobilen Phase ($CH_3OH/H_2O/H_3PO_4$ 85%ig; 50:50:1) gelöst. 20–40 µl der Lösung werden in das HPLC-System (Perkin-Elmer 12 cm × 4 mm Nucleosil C_{18} on 7,5 µ silica) injiziert. – Arzneim.-Forsch./Drug Res. **37(II),** 1396–1399 (1987). Zentrallab. Deutsch. Apotheker, Eschborn (D) K. Söllner

Fluorimetric determination of levoprotiline in human plasma after thin-layer chromatographic or high-performance liquid chromatographic separation. C. Horne, H. Spahn and E. Mutschler.

The quantitative determination of the new antidepressant drug levoprotiline, the R-(–)-enantiomer of oxaprotiline (α((methylamino)-methyl)-9,10-ethanoanthracene-9(10H)-ethanol), in human biological material is described. Analysis is performed by alkaline extraction with n-heptane-isopropanol, subsequent fluorescence derivatisation, with NBD chloride (4-chloro-7-nitrobenzofurazan), TLC or HPLC and fluorimetric measurement of the derivatisation product (λ_{max} ex. = 470 nm, λ_{max} em. = 525 nm). The sensitivity of the procedure (detection limit < 1 ng/ml) permits the performance of pharmacokinetic studies after therapeutic doses. – Arzneim.Forsch./Drug. Res. **37,** 1179–1181 (1987). Pharmakol. Inst. Naturwiss., Univ., Frankfurt (D)

Gas chromatographic-mass spectrometric determination of haloperidol in plasma. Application to pharmacokinetics. N. Häring, Z. Salama, L. Todesko and H. Jaeger.

A fused silica bonded phase capillary column, combined with negative ion chemical ionization (NICI), ammonia as a selective reagent gas and the monitoring of preselected characteristic ions (SIM), provide the combined sensitivity and selectivity necessary for reliable measurements in the low ng/ml range. The lower limit of detection was 0.1 ng/ml plasma and the calibration curve linear in the measured range of 0.1–5 ng/ml. In combination with the excellent imprecision and inaccuracy data and a recovery exceeding 90%, the method is very well suited for quantitative determinations of plasma samples generated during clinical studies, e.g. evaluating the pharmacokinetics and/or bioavailability/bioequivalence of haloperidol. – Arzneim.Forsch./Drug Res. **37,** 1402–1404 (1987). Anal. Res. Cent., LAB GmbH, Neu-Ulm (D)

Rapid and sensitive high-performance liquid chromatographic determination of bisoprolol in plasma and urine. J.-M. Poirier, M. Perez, G. Cheymol and P. Jaillon.

Ein einfaches und schnelles HPLC-Verfahren zur quantitativen Bestimmung von Bisoprolol, einem neuen hochselektiven β1-Adrenorezeptorantagonisten, aus Plasma und Urin wird beschrieben. Dazu werden die Proben mit einem geeigneten Standard versetzt (Struktur ist im Vergleich zur Bisoprololstruktur abgebildet), mit NaOH alkalisch gemacht und mit Diethylether extrahiert. Dann wird in 1 M Essigsäure rückextrahiert und die saure Phase auf eine µBondapak C_{18}-Säule injiziert, die mit Acetonitril/Methanol/0,09 M Phosphorsäure/Wasser (24:20:6:50) als mobiler Phase betrieben wird. Der Nachweis erfolgt fluorimetrisch bei 232/300 nm. Die Nachweisgrenze liegt bei 10 ng Bisoprolol/ml Urin. – J. Chromatogr. **426,** 431–437 (1988). Dept. Pharmacol., Saint-Antoine Hosp., Paris (F) R.H.S.

Liquid chromatographic method for the simultaneous determination of sulfasalazine and its metabolites in biological fluids. K. Róna, V. Winkler, T. Riesz and B. Gachályi.

An HPLC procedure was developed for the simultaneous determination of salicylazosulfapyridine, and its main metabolites 5-aminosalicylic acid and sulfapyridine, in human serum and synovial fluid. The analytical procedure consisted of a single ion-pair extraction step for an Extrelut column with methylene chloride. The investigated compounds and the added sulfadimidine internal standard were eluated from a Hypersil-MOS reversed-phase column by stepwise gradient; mobile phase was methanol/0.01 M potassium dihydrogenphosphate (3:7, 0.0–2.0 min and 8:2, 2.1–6.5 min). – Chromatographia **24,** 720–724 (1987). Postgrad. Med. Univ., I. Dept. Med., Budapest (H)

RP-HPLC assay for 1,2-5,6-dianhydro-3,4-disuccinyl-galactitol and its metabolites in blood plasma and liver. Gy. Szókán, I. Elekes, E. Táborhegyi, Gy. Csanádi and J. Bencze.

A method involving pre-column derivatization and HPLC assay is described for measuring submicrogram quantities of 1,2-5,6-dianhydro-3,4-disuccinyl-galactitol (1,2-5,6-dianhydro-3,4-bis(carboxypropionyl)-galactitol), an effective cytostatic drug and its metabolites in blood plasma and liver homogenate. The substance and its metabolites were derivatized with sodium pentamethylene-dithiocarbamate to form different bis(dithiocarbamoyl) esters, which can be detected by UV absorbance at 254 and 280 nm. The directly derivatized products were then extracted into $CHCl_3$, and after sample preparation resolved by RP-HPLC on SAS-Hypersil column. – Chromatographia **24,** 839–841 (1987). Inst. Org. Chem., Eötvös Univ., Budapest (H)

Determination of suramin in plasma and urine by ion-paired reverse-phase high-performance liquid chromatography. T.J. Stolzer, G. LaFollette, J. Gambertoglio, F. Aweeka and E.T. Lin.

Es wird eine einfache Analysemethode zur quantitativen Bestimmung der antiviral wirksamen Verbindung Suramin (SUR) im Plasma und Urin von AIDS-Patienten beschrieben. Die Proteine des Plasmas (0,5 ml) werden nach Zugabe von internem Standard (8 µg Kongorot (CR) in 50 µl Acetonitril) und 100 µl 0,5 M Tetrabutylammoniumchlorid (TBACl) mit 1 ml Acetonitril ausgefällt. Nach Zentrifugation wird der Überstand entnommen und der Niederschlag noch 2× mit 100 µl

TBACl-Lösung und 1 ml Acetonitril nachextrahiert. Die vereinigten Überstände werden mit Stickstoff auf ca. 1 ml eingeengt, davon werden 12 µl in den Hochdruckflüssigkeitschromatographen injiziert. Urine (0,5 ml) werden nach Zugabe von 50 µl internem Standard und 1 ml Acetonitril direkt injiziert. Die HPLC erfolgt an Säulen mit Nova-Pak C_{18}, 4 µm (Waters). Als mobile Phase dient Acetonitril/8,3 mM Ammoniumacetat-Puffer (pH 6,8) mit 4,3 mM TBACl (2:3); Fließgeschw. 1,0 ml/min; Meßwellenlänge 254 nm. Die Eichgeraden (Peakhöhenverhältnis SUR/CR gegen die SUR-Konzentration) zeigen bis 200 µg/ml Linearität, die untere Erfassungsgrenze liegt bei 2 µg/ml. – J. Liquid Chromatogr. **10,** 3451–3462 (1987). Div. Clin. Pharm., Univ., San Francisco, CA (USA)
W.H. Mennicke

High-performance liquid chromatographic analysis of azlocillin in serum. T. Valenza and P. Rosselli.

A rapid and sensitive HPLC method is described for the determination of azlocillin in serum. This method involves a short manual protein precipitation of the sample followed by an injection into a PR 18 column for separation and quantitation. The mobile phase was 22% (V/V) solution acetonitrile in phosphate buffer pH 4.8 at a flow rate of 2,5 ml/min. The spectrophotometer detector was set at 220 nm with a sensitivity of 0.08 AUFS. – Chromatographia **24,** 862–864 (1987). Clin. Lab., Anal., Careggi Hosp., Florence (I)

HPLC and RIA procedure for Cyclosporine A determination compared with clinical background. J. Klima, R. Petrásek, V. Kočandrle and J. Kašlik.

The aim of our study was to monitor Cyclosporine A (CA) during a long-term period and to determine whether the whole-blood levels, estimated by HPLC and radioimmunoassay (RIA), provide a relevant indicator of the toxic effect of CA, of the interference of endogenous substances with CA and of interactions of erythromycin and ketoconazole with CA metabolims. The ratios of CA concentrations, estimated by RIA and by HPLC were calculated in order to analyse changes in CA metabolism. The comparison of CA whole-blood levels estimated using HPLC and RIA makes it possible to analyse the individual biotransformation of CA to its metabolites, and interference of endogenous substance in special clinical situations. – Chromatographia **24,** 854–856 (1987). Inst. Clin. Exp. Med., Prague 4 – Krč (CS)

High-performance liquid chromatography of erythromycin propionyl ester and erythromycin base in biological fluids. C. Stubbs and I. Kanfer.

The simultaneous determination of erythromycin propionate and erythromycin base in serum and urine by high-performance liquid chromatography using oleandomycin as internal standard is described. The separation was achieved on a reversed-phase C_{18} column employing acetonitrile-0.05 M phosphate buffer (65:35), adjusted to pH 7.0 as the mobile phase with coulometric detection. Hydrolysis of the ester during blood sample collection was minimised by immediate high-speed centrifugation of collected blood samples, followed by separation and immediate freezing of the serum fraction. A solid-phase extraction procedure, combined with a simple phase-separation step was used prior to chromatographic analysis. The method has the necessary precision, sensitivity and accuracy to allow the simultaneous determination of both components in serum and urine following a single 500-mg oral dose of erythromycin estolate. – J. Chromatogr. **427,** 93–101 (1988). School Pharm. Sci., Rhodes Univ., Grahamstown (ZA)

Determination of 2′-acetyl erythromycin and erythromycin in plasma by HPLC using manual and robotic sample preparation. P. Kokkonen, H. Haataja and S. Välttilä.

A simple and specific HPLC method with coulometric detection was developed for the determination of 2′-acetyl erythromycin and erythromycin in human plasma. Methyl tert-butyl ether was used as the extraction solvent after alkalization of plasma samples. The plasma extracts were chromatographed on a reverse phase column using 4-component mobile phase. The manual sample preparation procedure was modified so that it could also be applied to the robotic system (Zymate™ Laboratory Automation System). The linear range was 0.25–7.0 µg/ml. The quantitation limit for 2′-acetyl erythromycin and erythromycin was 0.05 µg/ml. Equivalent manual and robotic sample preparation methods were used to analyze a large number of plasma samples. – Chromatographia **24,** 680–682 (1987). Orion Corp., Orion Pharmac., Espoo (SF)

Determination of nalbuphine by high-performance liquid chromatography with electrochemical detection: Application to clinical samples from post-operative patients. L.M. Dubé, N. Beaudoin, M. Lalande and I.J. McGilveray.

A rapid, selective and reproducible high-performance liquid chromatographic assay with electrochemical detection was developed for the determination of nalbuphine in human plasma. The method involves extraction with chloroform-isopropanol at pH 9.4, back-extraction into dilute phosphoric acid and reversed-phase chromatography on a µBondapak phenyl column. The recovery of nalbuphine and naltrexone (internal standard) was greater than 90%. Calibration curves were linear over a concentration range of 3–36 ng/ml with coefficients of variation, not exceeding 8%. Although the limit of detection was 0.3 ng/ml, the reliable limit of quantitation was 1 ng/ml (coefficient of variation 12%) using 1 ml of plasma. The dual-electrode detector was operated in the screening mode of oxidation (electrode 1, 0.3 V and electrode 2, 0.6 V), providing a greater specificity and reducing background noise. – J. Chromatogr. **427,** 113–120 (1988). Pharm. Chem. Div., Bureau Drug Res., Tunney's Pasture, Ottawa, Ontario (CDN)

Systematic analysis of solvents and other volatile substances by gas chromatography. J.P. Franke, J. Wijsbeek, R.A. de Zeeuw, M.R. Möller and H. Niermeyer.

Four column packings for screening volatiles in biological material by GC are evaluated. Retention data are standardized by the calculation of retention indices, and packing materials are compared by discriminating power and identification power. A combination of 5% Carbowax 20M on Carbopack B and 0.3% Carbowax 20M on Carbopack C appears to be best suited for screening. Hydroxy-*n*-alkanes are used as reference substances for the calculation of retention indices. – J. Anal. Toxicol. **12,** 20–24 (1988). Dept. Anal. Chem. Toxicol., State Univ., Groningen (NL); Inst. Rechtsmed., Univ., Homburg/Saar (D)

Analysis of thiodiglycol in urine of victims of an alleged attack with mustard gas, Part II. E.R.J. Wils, A.G. Hulst and J. van Laar.

Improvements on a procedure for the determination of thiodiglycol in urine are presented. This procedure is based on the conversion of thiodiglycol to mustard gas with concentrated HCl followed by headspace analysis. With deuterated thiodiglycol as the internal standard, more accurate quantitative analyses are possible. Residual amounts of chlorine in the water used for preparation of standard solutions posed problems, and the reaction between chlorine and thiodiglycol in water has been studied. The possible formation of mustard gas from thiodiglycol and sodium chloride was also investigated. The modified procedure was applied to urine samples of several Iranian patients who were victims of an alleged attack with mustard gas. With the exception of one relatively high value (330 ng/ml), the thiodiglycol concentrations were in the same range (10 to 100 ng/ml) as those found during an investigation in 1984. The urine of 20 male controls contained thiodiglycol amounts not above 20 ng/ml. – J. Anal. Toxicol. **12,** 15–19 (1988). Prins Maurits Lab., TNO, Rijswijk (NL)

A GS/MS method for the determination of 4,4′-diaminodiphenylmethane and substituted analogues in urine. J. Cocker, L.C. Brown, H.K. Wilson and K. Rollins.

A sensitive and specific GC/MS method has been developed for the analysis of 4,4′-diaminodiphenylmethane (DDM), 3-ethyl DDM (EDDM), and 3,3′-diethyl DDM (DEDDM) in urine. The method has been applied to the analysis of urine samples from workers exposed to a mixture of all three compounds, and the analysis has shown that EDDM and DEDDM are excreted in urine. We have also shown that there are two classes of conjugates present in urine. EDDM and DEDDM are excreted as heat labile conjugates, while DDM and EDDM are excreted, at least in part, as heat stable but alkaline hydrolyzable conjugates. It is proposed that the method described here could be used for biological monitoring of workers exposed to mixtures of DDM, EDDM, and DEDDM. – J. Anal. Toxicol. **12,** 9–14 (1988). Occupat. Med. Hyg. Labs., Health Safety Exec., London (GB)