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REVIEW

DRUG LEVEL MONITORING: CYTOSTATICS

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1. INTRODUCTION

Anticancer drugs have during the last decade become increasingly important in the treatment of neoplastic diseases. However, their use has been hampered by the high degree of serious side-effects observed, underlining a need for individualization of therapy. The demand for individualized cytostatic therapy is further underlined by the fact that most cancer patients exhibit changes in drug disposition, due not only to disturbances in liver or renal functions, but also to malnutrition and changes in plasma protein composition.

Most cancer patients treated by chemotherapy are given various combinations of cytostatics, which makes the pharmacokinetics of the individual drugs even less predictable. The pharmacokinetics of cytostatics are, moreover, sometimes time-dependent, i.e. change with the total dose and number of treatments.

Dose-dependent pharmacokinetics have been reported more frequently for anticancer drugs than for other drugs, probably because anticancer drugs are studied over a wide range of doses during early evaluation. Also in clinical practical a wide range of drug doses is routinely used. Very high doses of anticancer drugs are used in combination with marrow transplantation or administration of antidotes. The toxicity of some cytostatics can be reduced by dose fractionation, i.e. a low dose of the drug is administered, at frequent intervals.

Compared to other drugs more cytostatics need to be biotransformed, either intrahepatically or within the tumour cells, prior to exerting their biological activity. A multitude of metabolites with various degrees of cytostatic activity are often formed.

Quantification of systemic concentrations of cytostatics and their active metabolites will give little indication of their antitumour activity. One might expect a better correlation between blood or plasma levels and the toxicity, like nausea, vomiting, alopecia and myelosuppression. This situation is in contrast to what has been observed from traditional drug level monitoring, where correlations between blood or plasma levels of drugs and therapeutic efficiency as well as side-effects are used for optimization of the doses and treatment schedules, e.g. in the treatment of epilepsy and infectious diseases.

Many factors will modify the action of cytostatic drugs on the malignant cells. The variation in size, composition and blood supply of the tumours in combination with factors like penetration of the drugs into and out of the cells underline the difficulties in using systemic concentrations of cytostatics as a means of predicting therapeutic efficiency. Active metabolites are sometimes

formed and trapped within the tumour cells and are thus not available for quantification in plasma. Measurements of concentration—time profiles of the drug and active metabolites within the tumour or even within the cancer cell itself might be a more reliable parameter to correlate with the cytostatic activity.

Knowledge of intratumoural and intracellular concentrations of cytostatics is also of interest for the transfer of results from in vitro test systems to the clinical use of cytostatics.

The route of administration is an important factor with regard to access of a drug to different organs. Intravenously injected drugs have easiest access to most tissues. Orally administered cytostatic drugs must cross the gastrointestinal mucosa and the liver before reaching the general circulation. Wide variations of absorption have been described for a number of drugs. The bioavailability may be dependent upon food intake as well as first-pass hepatic metabolism. Anatomical abnormalities of the gastrointestinal tract are frequent in cancer patients, and this can modify drug absorption.

The main advantage of intraarterial administration of cytostatic drugs, compared to intravenous administration, is established during the first passage through the tumour; thereafter the drug returns to the systemic circulation and is distributed in the same way as after peripheral intravenous administration. By plasma pharmacokinetics only can the advantage of a reduced systemic delivery be evaluated. It should be emphasized that a lack of difference in plasma pharmacokinetics after intravenous and intraarterial administration should not be interpreted as a lack of pharmacodynamic difference.

Systematic studies of plasma pharmacokinetics after locoregional administration of cytostatics, e.g. intravesical, intrapleural and intraperitoneal administration, have resulted in highly efficient regimes almost without systemic toxicity.

A change in the administration time for intravenous administration changes the toxic pattern of many cytostatics. For compounds with linear pharmacokinetics it is possible to decrease the maximum plasma concentration without affecting the area under the plasma concentration—time curve (AUC). In such cases studies of the plasma pharmacokinetics can be useful to optimize the infusion time with respect to side-effects. For example, side-effects correlated with maximum plasma concentration of adriamycin include cardiac toxicity, nausea and vomiting. A more than three-fold increase in the cumulative dose has been possible by increasing the infusion time from < 3 min to > 24 h.

Proper handling of the biological samples is of a great importance to avoid metabolic and chemical changes of the drugs and metabolites after sampling. Due to the high chemical reactivity of many cytostatics the conditions for proper storage of the samples should be carefully evaluated.

Up to now more than 700 000 substances have been tested for cytostatic activity, of which fewer than 50 are in clinical use. For the majority of the cytostatic drugs used only scant information about their physical and chemical properties such as stability, lipophilic character, etc., as well as basic knowledge of their pharmacokinetics is available. The use of modern analytical techniques, including gas chromatograply (GC) and liquid chromatography (LC), with possibilities for sensitive and selective measurements, will provide a rationale for the more efficient use of new and old cytostatic drugs.

The use of liquid column chromatography for the analysis of antitumour antibiotics has recently been reviewed [1]. The present review concentrates mainly on the quantification of intact drugs. In cases where only a limited number of active metabolites is formed, techniques for their determination have been included. In general microbiological methods have not been dealt with.

2. ALKYLATING AGENTS

The alkylating anticancer drugs are assumed to exert their pharmacological effect by chemical reaction with vital cellular components. Great care must be exercised when developing analytical techniques since the compounds are susceptible to decomposition during storage of the biological samples, during the work-up procedure and during the chromatographic separation step.

Early studies on the pharmacokinetics of alkylating agents were performed after administration of radiolabelled drug followed by measurements of total radioactivity in plasma. The data obtained using these techniques must be interpreted cautiously since they only make possible codetermination of the parent compound, metabolites and chemical degradation products. The introduction of chromatographic techniques in the determination of alkylating agents has in many cases lead to a reevaluation of the pharmacokinetics of the compounds.

2.1. Chlorambucil

Chlorambucil is used for the treatment of malignant lymphomas and chronic lymphocytic leukaemia. In man the drug is extensively metabolized to phenylacetic acid mustard [2—5]. Studies using human cell lines in vitro have shown the metabolite to have similar cytotoxic activity to chlorambucil [6]. It is therefore important that both the parent compound and the metabolite are determined when trying to correlate plasma concentrations with antineoplastic activity.

Chlorambucil is rapidly decomposed in aqueous solution, the stability being influenced by pH, temperature and chloride ion concentration [7—9]. The stability in plasma is higher [10, 11] due to the fact that chlorambucil is extensively bound to albumin and the compound is considerably more stable when bound to albumin than unbound in solution [11].

2.1.1. Gas chromatography

GC with selected-ion monitoring (SIM) [12—14] seems to give higher sensitivity and selectivity compared to methods based on liquid column chromatography. However, the samples have to be derivatized prior to the GC step. Both alkylation [12, 14] and silylation [13] reactions have been utilized for the derivatization of the carboxylic group. To increase the chemical stability of the nitrogen mustard group it has been converted to a thiazane by reaction with sodium sulphide [5, 14]. The limit of determination (C.V. $< 10\%$) was 2—5 ng/ml both for chlorambucil and phenylacetic acid mustard [5].

2.1.2. Liquid column chromatography

Reversed-phase liquid column chromatography with photometric detection has in several cases been used to quantify chlorambucil and phenylacetic acid mustard [15—18]. However, most techniques seem to lack the sensitivity needed for meaningful pharmacokinetic studies after oral administration of therapeutic doses of chlorambucil. The method of Leff and Bardsley [15] comprises several extraction steps and esterification of the carboxylic group of chorambucil prior to analysis, and the technique has not been applied for the determination of phenylacetic acid mustard. Direct injection of plasma (about 30 μl) into the chromatographic system equipped with a guard column has been used by Zakaria and Brown [18]. However, it is difficult to draw any definitive conclusions concerning the usefulness of the method for quantification at the low ng/ml levels.

2.2. Melphalan

Melphalan is used for the treatment of multiple myeloma, malignant melanoma and carcinoma of the ovary. Melphalan readily undergoes hydrolysis in vitro, the hydrolysis rate being dependent on pH, chloride ion concentration, bile acids and albumin concentration [19—25].

2.2.1. Liquid column chromatography

Liquid column chromatography with photometric detection has been widely used for the determination of melphalan in biological samples [19, 26—28]. The early methods had a limit of detection of about 50 ng/ml in human plasma which is insufficient for measurements of levels after normal therapeutic doses of oral melphalan. An increased sensitivity (5 ng/ml) has been obtained using a more selective work-up procedure (XAD-2) and a chromatographic system with a higher separating efficiency [28]. Similar sensitivity has also been obtained using LC with fluorometric detection of melphalan [29].

2.2.2. Selected-ion monitoring

Analysis of melphalan by SIM has only been utilized in a few instances [30, 31]. Tattersall et al. [30] used the direct insertion probe after purification of diazomethylated melphalan by thin-layer chromatography (TLC). Pallante et al. [31] used GC in combination with SIM after trifluoroacetylation of melphalan followed by esterification with diazomethane, permitting quantification of about 2 ng/ml of plasma.

2.3. Busulfan

Busulfan is almost exclusively used for the treatment of chronic myelocytic leukaemia. Busulfan is more stable than the nitrogen mustards chlorambucil and melphalan in aqueous solution [32] but, contrary to the nitrogen mustards, a decreased stability is observed in plasma [33]. Early studies on the fate of the drug in humans used radiolabelled drug followed by measurements of total radioactivity in plasma and urine [34]. The only techniques with sufficient sensitivity and selectivity for determination of busulfan after

therapeutic doses are based on GC. Busulfan is converted to 1,4-diiodobutane by reaction with sodium iodide and the quantification is performed either by SIM [35] or electron-capture detection (ECD) [36]. Both techniques allow determinations of about 5 ng/ml of plasma.

2.4. Cyclophosphamide

Cyclophosphamide is one of the most frequently used alkylating agents in cancer chemotherapy due to its wide spectrum of activity and its high therapeutic index. The parent compound is pharmacologically inactive and the biological effects are mediated by compounds generated by metabolism of the drug (Fig. 1). The metabolism of cyclophosphamide has recently been reviewed [37, 38]. The initial step of activation is the formation of 4-hydroxycyclophosphamide, which is in equilibrium with an open-ring aldehyde, aldophosphamide. This compound spontaneously eliminates acrolein and yields phosphoramide mustard which is the ultimate alkylating metabolite. 4-Ketocyclophosphamide and carboxyphosphamide are devoid of antitumour activity and appear to be detoxification products. Nornitrogen mustard is supposed to have insignificant cytotoxic effect in vivo.

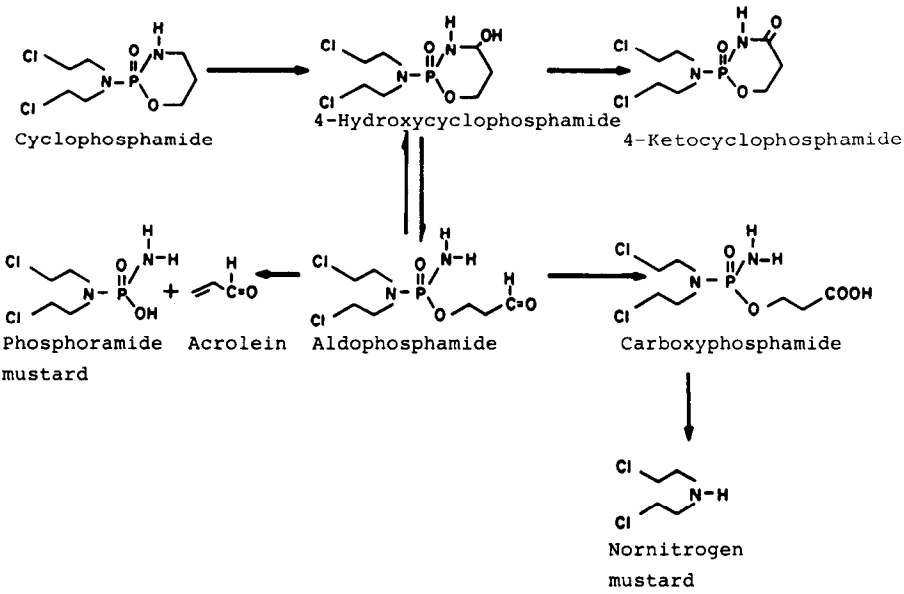

Fig. 1. Metabolism of cyclophosphamide.

2.4.1. Gas chromatography

It was soon observed that cyclophosphamide partly decomposes in the GC system giving a dehydrohalogenated compound formed by an intramolecular alkylation process [39]. Facchinetti et al. [40] and De Bruin et al. [41] used the nitrogen–phosphorus detector for the determination of cyclophosphamide in plasma but it is unclear if the quantification was performed on intact cyclophosphamide or its decomposition product.

By proper choice of injection and column temperature Van den Bosch and

De Vos [42] assumed that cyclophosphamide was quantitatively converted to its dehydrohalogenated compound. Most GC techniques are based upon prior derivatization of cyclophosphamide using trifluoroacetic [43—47] or heptafluorobutyric [48] anhydride. The quantification has been performed by ECD [49], nitrogen—phosphorus detection (NPD) [44, 45, 47, 48] or SIM [43, 46]. The latter technique offers the highest sensitivity with a lower limit of detection of about 3 ng/ml in plasma and urine [46].

The cytotoxic metabolite phosphoramide mustard and nornitrogen mustard have been determined using NPD [48] or SIM [46]. Both techniques are based upon trifluoroacetylation of nornitrogen mustard. Juma et al. [47] determined phosphoramide mustard after methylation followed by trifluoroacetylation which gives a higher derivatization yield than the diazomethylation procedure utilized by Jardine et al. [46]. The incomplete derivatization is compensated for by the use of a stable-isotope-labelled internal standard. The sensitivity of the techniques is comparable (30—40 ng/ml) with regard to phosphoramide mustard while SIM has about ten times higher sensitivity when determining nornitrogen mustard. The risk for artifacts formed during the work-up procedure has been thoroughly studied by Jardine et al. [46]. Significant amounts of 4-ketocyclophosphamide and carboxyphosphamide decompose to nornitrogen mustard during the extraction procedure, which will cause the levels of nornitrogen mustard to be artifactually high.

2.4.2. Liquid column chromatography

Reversed-phase liquid column chromatography has been used for the analysis of cyclophosphamide in the presence of its hydrolysis products using photometric detection at 200 nm [50]. The technique lacks the sensitivity necessary for determination of the drug in biological samples.

2.4.3. Mass spectrometry

The concentration of cyclophosphamide in blood and urine has been determined by direct-insertion electron-impact mass spectrometry (EI-MS) using deuterium-labelled cyclophosphamide as internal standard [51]. The technique allows determination down to about 1 μg/ml. Interferences from endogenous material become severe at lower concentrations and further purification of the extracts is necessary. The direct-insertion technique has also been utilized for pharmacokinetic studies of the enantiomers of cyclophosphamide and studies on the formation of 4-ketocyclophosphamide and carboxyphosphamide. The enantiomers have either been administered separately [52] or have been given as a pseudoracemate in which one of the enantiomers has been labelled with deuterium [53, 54]. The quantification of the metabolites requires purification of the biological samples by TLC followed by derivatization.

Field-desorption mass spectrometry (FD-MS) has been utilized for the determination of cyclophosphamide in urine, serum and cerebrospinal fluid using a stable-isotope-labelled internal standard [55]. Thorough purification of the biological samples by LC is necessary to obtain sufficiently high sensitivity.

2.5. Thio-TEPA

The alkylating agent, N,N′,N″-triethylenethiophosphoramide (thio-TEPA) is mainly used for local treatment of superficial bladder tumours by intravesical instillation. It is metabolized to N,N′,N″-triethylenephosphoramide (TEPA).

Photometric determination of thio-TEPA in plasma is based on reaction with *p*-nitrobenzylpyridine [56, 57]. Thio-TEPA is extracted with diethyl ether, and the organic extract is reacted with *p*-nitrobenzylpyridine. The reaction product is dissolved and quantitated at alkaline pH by photometry (540 nm). The sensitivity of the method is 2 μg/ml. This method does not distinguish between thio-TEPA and TEPA.

For fluorometric determination, thio-TEPA and TEPA were separated by solvent extraction and reacted with β-naphthol. The formed products were isolated and quantified by fluorometric measurements (290 nm/355 nm). The recovery of thio-TEPA in plasma and urine in the range 50—200 ng/ml was 95 ± 20% [58].

At the moment no chromatographic techniques are available for this drug.

3. ANTIMETABOLITES

3.1. Methotrexate

Methotrexate (amethopterin, 4-amino-N^{10}-methylpteroylglutamic acid) is an antifolate that has been used clinically since 1953. It has significant antitumour activity in acute leukaemia and several other neoplastic diseases. In addition, it has an important use in the treatment of diseases such as psoriasis, sarcoidosis and Wegener's granulomatosis. Until 1967 it was routinely used in doses of less than 1 mg/kg. Currently, "high-dose" methotrexate up to 500 mg/kg is being used. However, high-dose methotrexate therapy is accompanied by acute folate stress, which may be life-threatening. Citrovorum factor (folinic acid) is used to protect the patient from these effects. In theory, citrovorum factor should be administered immediately after circulating methotrexate has fallen below the effective level of tumour cell kill and be maintained until normal cells begin to recover. The clinical importance of high-dose methotrexate in combination with citrovorum rescue might be reflected by the vast numbers of methods for the determination of methotrexate in plasma and serum.

The metabolism of methotrexate is dose-dependent. It is apparently not significantly metabolized in man following the administration of conventional doses. However, it has been shown that man and primates both excrete substantial quantities of 7-hydroxymethotrexate when they are placed on high-dose regimen (> 50 mg/kg). This metabolite is less water-soluble than the parent compound and may cause renal damage due to crystallization in the tubules. Methotrexate is also converted to 4-amino-4-deoxy-N^{10}-methylpteroic acid (DAMPA) following cleavage of the pteorate—glutamate peptide bond by the carboxypeptidases of some intestinal bacteria. A further metabolite, 7-hydroxy-DAMPA, has also been found. Following passage into cells, methotrexate is converted to a series of polyglutamates.

Dosage forms of methotrexate may contain up to 15% of impurities, the amount of impurities present being dependent on the particular manufacturing process. The febrile reaction which occurs after intrathecal injection of methotrexate has been attributed to the presence of impurities and/or degradation products, some resulting from photodecomposition of methotrexate.

In conclusion, there is a great need for a selective and sensitive method for methotrexate analysis in biological samples as well as for product control. Techniques for the measurement of methotrexate in biological samples have recently been reviewed [59].

3.1.1. Non-chromatographic procedures

3.1.1.1. Fluorometry. The method reported by Freeman [60] is based on oxidation of methotrexate with permanganate to yield a strongly fluorescent product. The method involves the measurement of fluorescence before and after oxidation, the difference of two fluorescence readings being proportional to the concentration of methotrexate. The sensitivity of the procedure is reported to be 5 ng/ml, but it has been claimed [61, 62] that it is impossible to reproduce this method for such low concentrations. By the introduction of an improved work-up procedure [61] only a single fluorescence reading after oxidation was necessary. The selectivity of the fluorometric methods for the determination of methotrexate was to some extent improved by the introduction of an extraction procedure [62].

In conclusion, the fluorometric techniques lack selectivity because most other pteridines such as citrovorum factor contribute. The lack of selectivity makes the fluorometric methods unsuitable for the determination of methotrexate in urine samples.

3.1.1.2. Radioimmunoassay. A large number of radioimmunoassays (RIA) for the quantification of methotrexate in plasma have been presented [59, 63—75]. They offer fast and sensitive (approx. 1 ng/ml) methods for measuring the drug, and are especially useful when large batches of samples are involved. The disadvantage of the high sensitivity and the narrow range of standards is that high serial dilutions are necessary for most samples and, in many cases, repeated assays have to be carried out.

Tritiated methotrexate has mainly been used as the labelled moiety for the RIA. The assays in which γ-emitters (^{75}Se and ^{125}I) are used have significant practical advantage over the β-emitter [73], and are much better suited to automation and clinical application. The main advantage of ^{75}Se-labelled methotrexate is its longer half-life, 121 days, compared with 60 days for ^{125}I, thus giving the ^{75}Se-labelled methotrexate a much longer shelf-life [73]. A fully automated, continuous-flow RIA procedure for methotrexate, using ^{125}I, has been presented [75], permitting precise (coefficient of variation C.V. $< 2.5\%$) determinations of 30 samples per h.

Interference of DAMPA with the RIA of methotrexate has been reported [76], and as a consequence plasma samples collected in the terminal phase of an elimination curve may give falsely high results. The risk of cross-reactivity with other methotrexate analogues and metabolites should also be considered.

3.1.1.3. Enzyme immunoassay. An enzyme immunoassay (EIA) for the determination of methotrexate [77] has been described, in which an enzyme—

methotrexate conjugate which is stable for long periods is used. The assay can be performed by the use of a spectrophotometer, and has a sensitivity of about 90 ng/ml. A fully mechanized EIA has also been described [78]. Although this sensitivity may be adequate for detection of toxic levels, the method cannot be used for pharmacokinetic studies.

3.1.1.4. Enzyme inhibition assay. The principle for the assay of methotrexate by enzymatic methods is based on the stoichiometric inhibition of the enzyme dihydrofolate reductase (DHFR), an enzyme whose activity is conveniently measured by following NADPH consumption spectrophotometrically [79—85]. Bacterial enzyme is reported to be more stable for this type of analysis than enzyme from mammalian liver. While methotrexate has a high affinity for DHFR, any other substance that binds to the enzyme, e.g. reduced folates or methotrexate metabolites, is theoretically able to interfere with the analysis. Interference by trimethorprim in the analysis of methotrexate by enzyme inhibition assay has been reported [86].

3.1.1.5. Competitive protein-binding assay. Competitive protein-binding assay is a technique suitable for both pharmacokinetic studies and clinical monitoring of methotrexate [87—90]. The assay is based on competition between [^{3}H]methotrexate and unlabelled methotrexate for DHFR, with a subsequent removal of unbound drug by charcoal adsorption. The absolute sensitivity of the radioassay is limited by the specific activity of the [^{3}H]-methotrexate and can be approximately 0.5—1 ng/ml drug in plasma samples. Folic acid, methyltetrahydrofolate, formyltetrahydrofolate, and dihydrofolate in physiological concentrations do not interfere [88].

3.1.2. Liquid column chromatography

The main advantage of the analytical procedures for the determination of methotrexate based on high-performance liquid chromatography (HPLC) is the high selectivity. These techniques give the possibility not only for the simultaneous determination of intact drug and metabolites in biological samples, but also for the characterization of purity and quality in the various pharmaceutical formulations [91—93]. HPLC is less suitable for the determination of the large numbers of biological samples that might be required in the routine treatment of patients with high doses of methotrexate and citrovorum rescue.

The lack of sensitivity for most of the HPLC methods hampers to some extent their use in clinical practice.

The work-up procedures for the determination of methotrexate and its metabolites by HPLC vary with the origin of the biological samples. For example, urine samples can be injected directly into the chromatograph after removal of particles by centrifugation or filtration [93—97], while plasma and serum samples require a more laborious work-up procedure.

The work-up procedures for the determination of methotrexate and metabolites in plasma and serum samples include protein denaturation and/or extraction, diafiltration and centrifugation followed by injection of the entire plasma sample. Considerable losses of the compounds of interest have been reported during the protein precipitation step. Yields of 40—70% are reported when trichloroacetic acid [98—101], perchloric acid [94, 102—104] or acetonitrile

[95] are used. Liquid—liquid extraction of the acidified plasma sample with ethyl acetate—isopropanol gave a recovery of 38% [105]. Column extraction using Sep-Pak [106] or ion-exchange columns [97] and diafiltration [107] seems to be more suitable for the isolation of methotrexate and its metabolites, giving recoveries of $\geqslant$ 85% for the intact drug. The direct injection technique for plasma samples [96, 108] is of a limited value, since only small amounts ($\leqslant$ 50 μl) can be injected into the chromatographic systems to maintain their performance, with a concomitant lack of sensitivity. This drawback was to some extent eliminated by preextraction of the samples with ether—butanol prior to injection of the sample [109].

The separation of methotrexate and its metabolites is based on reversed-phase chromatography with or without addition of ion-pairing agents in the mobile phase [94, 96—98, 101, 103—109]. Alternatively, cation- [95] or anion- [99, 100, 102] exchange chromatography can be used with almost the same separation efficiency and selectivity.

The use of photometric detection at 305—315 nm gives increased detection selectivity compared to photometric detection at 254 nm. The detection limit using photometric detection is about 50 ng/ml, but can be enhanced by a factor of 5 if an on-line column enrichment step [99, 100] is included. The electrochemical detector [105] (limit of detection 10 ng/ml of plasma) has the advantage of being somewhat more sensitive than the photometric detectors. Fluorometric detection of methotrexate after precolumn derivatization [98] (limit of detection 10 ng/ml) is also favourable compared to photometric detection of the intact drug with respect to sensitivity.

3.1.3. Isotachophoresis

One method for the determination of methotrexate in plasma by isotachophoresis has been published [110]. The tedious work-up procedure includes protein precipitation, removal of chloride ions, addition of silver nitrate and precipitation of the silver—methotrexate complex. After rinsing, drying and redissolving the complex the isotachophoresis is performed. The lower limit of detection is about 25 μg/ml of plasma.

3.1.4. Mass spectrometry

Methotrexate, and its degradation products as well as analogues have been characterized by the use of MS. Both field ionization [111], FD [107, 112] and conventional EI and CI techniques [113] have been used.

3.1.5. Method comparison

A great number of papers dealing with methods for the determination of methotrexate include a comparison of the analytical results obtained with different methods in order to evaluate accuracy and selectivity [59, 67, 73, 75, 77, 78, 82, 84, 90, 96, 104, 109, 110, 114—117]. The data are generally presented in scatter diagrams with calculated linear regression statistics. A high value for the regression coefficient ($r > 0.90$) is often falsely interpreted as a close agreement of the analytical results obtained by the two methods being compared. In fact, reevaluation according to the principles given in ref. 118 of method comparison data presented reveals the existence of largely deviating results for the analytical methods used.

3.2. 5-Fluorouracil

5-Fluorouracil (5-FU) is frequently used for the treatment of gastrointestinal, head and neck carcinomas. The cytotoxic effect of 5-FU requires metabolic activation to 5-fluoro-2′-deoxyuridine 5-monophosphate which inhibits the enzyme thymidylate synthetase. The cytotoxic effect is also assumed to be due to the formation of 5-fluorouridine triphosphate which is incorporated to form fraudulent RNA. 5-FU is metabolized in the liver to dihydro-5-FU which is devoid of cytotoxic activity. The active 5-FU nucleotides are trapped within the cell since they cannot readily cross the cell membranes due to their highly polar character.

3.2.1. Non-chromatographic procedures

3.2.1.1. Microbiological methods. Microbiological assays [119, 120] have high sensitivity (about 10 ng/ml) but may be unsuitable when the patients are also receiving antibiotics. The accuracy of the microbiological techniques when measuring low levels of 5-FU is questionable [121].

3.2.1.2. Photometry. Spectrophotometry of 5-FU in biological samples is only applicable for measurement of μg/ml concentrations [122, 123]. The method of Morimoto et al. [123] uses ion-pair extraction of 5-FU to methylene chloride followed by back-extraction into an aqueous solution. The selectivity of the method with regard to metabolites of 5-FU has not been evaluated.

3.2.2. Gas chromatography

GC of 5-FU is hampered by its polar character resulting in adsorption of the compound in the GC system. Chromatography of the underivatized compound on packed columns requires careful deactivation of the glass column by silanization followed by treatment with Carbowax 20 M and the use of a polar stationary phase (Versamide 900) coated on a resilanized packing material [124]. The use of capillary columns has been reported to minimize the adsorption phenomenon [125]. 5-FU has in most cases been chromatographed after prior derivatization and a multitude of derivatization reactions have been utilized. Methylation has been performed using flash alkylation with trimethylanilinium hydroxide [126—130], reaction with diazomethane [121, 131] or reaction with methyl iodide [132]. Homologous alkyl derivatives, e.g. butyl [133], pentyl [134] and hexyl [135, 136], have also been prepared. Silylation reactions have been used to convert 5-FU to its corresponding trimethylsilyl [137—143], or chloromethyldimethylsilyl derivative [144, 145].

GC with SIM [128, 130—134, 139—141] seems to offer the highest sensitivity and in several instances the limit of sensitivity has been about 1 ng/ml of plasma. Detection by the nitrogen—phosphorus detector [125, 129, 131] and the electron-capture detector [144, 145] offers similar sensitivity (10—100 ng/ml plasma) while detection by the flame ionization detector only seems to be applicable to plasma levels exceeding 200 ng/ml [135, 137, 138].

3.2.3. Liquid column chromatography

Reversed-phase liquid column chromatography using an acidic mobile phase has been widely used for the separation of 5-FU from endogenous material and metabolites formed by anabolic and catabolic activity [146—157]. The separa-

tion has in most instances been performed using μBondapak C_{18} packing material. In some instances adsorption chromatography on silica gel with organic solvents as eluents has been used [158, 159]. Anion-exchange chromatography using an alkaline eluent [160] or cation-exchange chromatography with a mobile phase of pH 3.5 [161] have also been utilized. However, in the latter case the ion-exchange mechanism can only insignificantly contribute to the retention of 5-FU since the compound is a weak acid (pK_a = 8.0). Peters et al. [162] used a porous polymer packing material for the separation of 5-FU and uracil originating from catabolic conversion of uridine which was administered in connection with 5-FU. The separation was carried out at high pH using cetyltrimethyl ammonium as ion-pair agent.

The quantification of 5-FU has been carried out using photometric detection at 254—280 nm. The sensitivity of the techniques is generally in the range 10—100 ng/ml of plasma. The highest sensitivity was obtained using an extensive work-up procedure comprising ion-exchange chromatography and solvent extraction, minimizing interferences from plasma constituents and allowing large plasma samples and high detector sensitivity to be used [148]. Techniques based on batch extraction of plasma using *n*-propanol—diethyl ether have a limit of sensitivity of about 25- 100 ng/ml of plasma [146, 147, 153].

3.2.4. Isotachophoresis

Isotachophoresis has been used by Gustavsson and co-workers [163, 164] for the determination of 5-FU in serum. After deproteinization and purification using an ion-exchange column, 5-FU is separated by isotachophoresis and quantification is performed by light transmission at 254 nm. The limit of quantification is about 7 ng/ml.

3.3. Cytosine arabinoside

Cytosine arabinoside (Ara-C) is extensively used in the treatment of acute myelocytic leukaemia. The active intracellular form of Ara-C is the triphosphate (Ara-CTP), while intact Ara-C has no pharmacologic activity. Ara-CTP exerts its effect by blocking DNA synthesis and possibly by introducing Ara-C moieties into the DNA coding sequences. In man, Ara-C is rapidly metabolized by deamination, mainly by the liver, to an inactive product, uracil arabinoside (Ara-U).

3.3.1. Non-chromatographic procedures

3.3.1.1. Biological methods. The biological methods for the determination of Ara-C [165, 166] utilize the inhibition of [^{3}H]thymidine incorporation into the DNA of murine leukaemia L1210 or suspended mouse spleen cells. The limit of detection in plasma is about 20 ng/ml. These methods have low selectivity with interference from other cytostatics present, and long incubation times ($\geq$ 5 h) are required.

3.3.1.2. Enzymatic methods. The enzymatic method for the estimation of plasma concentrations of Ara-C [167] consists of the phosphorylation of Ara-C with [γ-^{32}P]ATP in the presence of deoxycytidine kinase and Mg(II), with subsequent isolation of the 5'-monophosphate of Ara-C by TLC. The Ara-CMP

spot was assayed for radioactivity by liquid scintillation. The analytical procedure is time-consuming (> 3 h). The selectivity is low as deoxycytidine and other nucleosides, such as cytidine, deoxyadenosine and deoxyguanosine, interfere with the assay.

3.3.1.3. Spectrophotometry. The differential analysis of cyclocytidine, Ara-C, and Ara-U in plasma by UV spectrophotometry [168] is based on the resolution of a single UV curve into three component parts. The method does not require preliminary separation of the compounds. The work-up procedure consists of protein precipitation by heavy metals and filtration before the photometric readings. The sensitivity of the procedure is 2—5 μg/ml. The selectivity is low, with interference from other UV-absorbing components in plasma.

3.3.1.4. Radioimmunoassay. The sensitivity of the RIA methods for the determination of Ara-C and Ara-U in plasma is approximately 20 ng/ml [169—171]. By a proper choice of pH in the binding reaction of Ara-C with the antibody, cross-reactivity of Ara-U can be avoided [169]. By performing the RIA at both pH 6.2 and 8.6 it is even possible to use antibodies towards Ara-C for the simultaneous determination of Ara-C and Ara-U [169, 170]. The RIA methods are almost free from interference by deoxycytidine, cytidine and other nucleosides as well as from various antibiotics.

3.3.2. Gas chromatography

GC with SIM [172, 173] and with NPD [173] has been used for the determination of Ara-C in plasma. Both methods require time-consuming multiple steps of extraction and derivatization.

The sensitivity of the method described by Pantarotto et al. [172] is limited to 100 ng/ml of plasma due to a high background signal and the small plasma volumes used.

Boutagy and Harvey [173] compared protein precipitation with ethanol or trichloroacetic acid and ultrafiltration as work-up procedure for plasma samples. The recovery in the ultrafiltered plasma (85%) corresponds to the non-protein-bound fraction of Ara-C [174] and was superior to that obtained with the protein precipitation procedures. The subsequent derivatization step and extraction of the acetylated Ara-C were quantitative. The limit of detection was 40—70 ng/ml of plasma using NPD whilst GC—MS showed a greater selectivity with a detection limit of 1 ng/ml of plasma.

A comparison of the plasma pharmacokinetics based on concentrations determined by a biological method [165, 166] and GC—MS [173] clearly demonstrates the need for highly selective analytical procedures [175].

3.3.3. Liquid column chromatography

Early liquid column chromatographic methods for the determination of Ara-C and its metabolites suffer from lack of details of the analytical procedures used [176—181]. However, even though quantitative data are missing, they clearly demonstrate the possibilities of the LC technique to separate Ara-C and corresponding nucleosides and nucleotides from endogenous compounds.

The method presented by Bury and Keary [182] for the determination of

Ara-C has a sensitivity of 20 ng/ml of plasma using photometric detection. The work-up procedure used, i.e. precipitation of proteins with trichloroacetic acid, is reported to be quantitative. However, the front peak originating from endogenous plasma constituents was broad, allowing the measurement of Ara-C as a tailing peak only. Ara-U could not be determined by this method.

The determination of Ara-C and Ara-U in ultrafiltered plasma including reversed-phase HPLC separation with phosphate buffer pH 7 as a mobile phase is described in ref. 183. Photometric detection at 280 nm and 264 nm, respectively, gave a sensitivity of 2—10 ng/ml plasma.

The most convenient method for the quantification of Ara-C and Ara-U in plasma, urine and cerebrospinal fluid is based on a reversed-phase system similar to that given in ref. 183. To avoid conversion of Ara-C to Ara-U in collected blood samples, a deaminase inhibitor, tetrahydrouridine, is added prior to centrifugation. However, the need for and the effect of the deaminase inhibitor were not evaluated. Direct injection of the plasma fraction into the liquid chromatograph gave clean chromatograms. The coefficient of variation was less than 5% at 50 ng/ml.

A reversed-phase HPLC column switching system for the simultaneous quantification of Ara-C, Ara-U and sodium salicylate has recently been described [184]. Sodium salicylate was coadministered with Ara-C to enhance absorption from the rectum. This analytical method does not offer any advantages compared to that described in ref. 174, unless there is a need for the simultaneous quantification of sodium salicylate, since the work-up procedure is tedious. It comprises protein precipitation and evaporation of the supernatant followed by reconstitution with water prior to injection into the chromatographic system. The sensitivity was 60 ng/ml.

3.4. Purine antagonists

The purine antimetabolites 6-thioguanine (6-TG) and 6-mercaptopurine (6-MP) have found important application in the clinical chemotherapy of acute leukaemia. Both agents must be metabolized prior to exerting their antitumoral effect. Azathiopurine (Aza), in general regarded as a slow-release preparation of 6-MP, is widely employed for immunosuppressive therapy (e.g. in organ transplantation) but it is also used as an antineoplastic drug. In general, available methods for the determination of the purine antagonists in biological samples are focused on the inactive intact drugs only, which severely limits the value of the information obtained.

The proper handling of the samples to avoid metabolic and chemical conversion prior to and during the analysis has only recently been taken into consideration [185—189]. Oxidation of the thiol group was prevented by the presence of dithioerythritol [185—188]. 6-MP has also been converted to 6-mercaptopurine-N-ethylmaleimide to prevent autoxidation [189].

3.4.1. Non-chromatographic procedures

3.4.1.1. Spectrophotometry. A spectrophotometric method has been used for determination of microgram amounts of the 6-thiopurines in urine [190]. The method comprises column extraction using a cation-exchange column and

photometric evaluation of 6-MP and 6-thiouric acid (6-TUA) as the spectral changes obtained after the addition of mercuric ions to 6-thiopurines. Aza is determined after conversion to 6-MP by reaction with glutathione, the urinary concentration being calculated as the difference between urinary 6-MP concentrations before and after glutathione treatment. However, the method is time-consuming and unselective.

3.4.1.2. Fluorometry. Fluorometric methods have been used for the determination of 6-MP [191, 192], 6-TG [193, 194] and Aza [192]. All procedures include oxidation of the drugs to fluorescent products since the intact drugs have only limited fluorescence. Odixation with hydrogen peroxide in acetic acid [194] or with potassium permanganate—hydrogen peroxide [191, 193] gives highly fluorescent S-oxides and sulphonates of the 6-thiopurines. The work-up procedure for plasma samples includes only protein precipitation. This technique, however, is less reliable since considerable amounts (30—50%) of the drugs coprecipitate. High plasma blank readings, especially with uremic plasma, and the low selectivity limit the usefulness of these methods. The sensitivity of the methods is not sufficiently high for pharmacokinetic studies, the limit of detection varying from 50 ng/ml [191] to 4 μg/ml [193, 194].

As an alternative to protein precipitation, 6-MP can be extracted from plasma after conversion of 6-MP into a phenyl mercury derivative prior to re-extraction and oxidation with potassium chromate [192]. The sensitivity increased considerably compared to fluorometric methods using protein precipitation as work-up procedure, the limit of detection being 10 ng/ml. The method is reported to be highly selective, but interference from Aza is reported. In fact, Aza can be quantified in plasma using this method after hydrolysis to 6-MP.

3.4.2. Gas chromatography

6-Mercaptopurine in plasma samples has been determined by GC after alkylation [185, 193, 195, 196]. The methyl derivative, formed by "flash methylation" [172, 185] or by extractive methylation [195], is less suitable as one of the metabolic pathways of 6-MP is S-methylation, and this metabolite will hence be codetermined. The poor stability of this derivative also enhances the problems with quantitative determination of plasma levels of 6-MP by these methods. The reported sensitivity of the method based on methylation of 6-MP was 0.5 μg/ml using flame ionization detection (FID) [185] and 20 ng/ml using SIM [195].

The method presented by Floberg et al. [196] has a sensitivity of 2 ng/ml. The analytical procedure involves extraction and derivatization directly from plasma samples by extractive alkylation with pentafluorobenzyl bromide and determination by SIM. The selectivity of the method to Aza and metabolites was carefully evaluated.

3.4.3. Liquid column chromatography

An assay for azathiopurine and 6-mercaptopurine by reversed-phase HPLC has been presented by Fell et al. [197]. This method has not been applied to quantification of the drugs in biological samples.

A great number of papers deal with the isolation, separation and identification of the purine antagonists and their metabolites in biological samples

[198—205]. The complex metabolic pattern of the purine antagonists is hence well understood, although this knowledge has not been utilized in clinical practice.

Liquid column chromatography has been used for quantification of 6-MP [186—189, 206—209], 6-TG [207, 210, 211] and Aza [186, 189, 212, 213].

The liquid column chromatographic methods for quantification of the purine antagonists in biological samples differ mainly in the work-up procedure. Precipitation of plasma proteins with trichloroacetic acid [187, 206], perchloric acid [207, 211] or acetonitrile [194, 208] is less suitable because of considerable losses of the drugs and metabolites. Isolation of the purine antagonists from biological samples by liquid—liquid extraction with ethyl acetate [186, 213] results in an almost complete recovery (> 98% [213]). The extraction method used in ref. 188, previously used as a purification step preceding fluorometric quantification [192], is highly selective.

Direct injection of plasma into a reversed-phase column for the determination of 6-MP is simple [209], but only small amounts of plasma samples can be injected to avoid disturbances in the chromatographic process, and as a consequence the sensitivity of the method using photometric detection is poor.

Ultrafiltration of plasma prior to injection onto a reversed-phase column is a technique that has been applied for the determination of 6-TG in plasma [210]. The chromatographic system used (a Nucleosil C_8 column with citrate—phosphate buffer as eluent) was very well suited for the separation of purine analogues. The sensitivity of the method using photometric detection was 200 ng/ml.

The simultaneous determination of Aza and 6-MP is described in ref. 189. By derivatization of 6-MP to 6-mercaptopurine-N-ethylmaleimide, this drug could be extracted together with Aza into ethyl acetate. The derivatization procedure also enabled reversed-phase HPLC separation with photometric detection at 280 nm.

The sensitivity of the liquid column chromatographic methods for the determination of the purine antimetabolites using photometric detection varies between 3 ng/ml [187] and 200 ng/ml [206, 209, 210], the variation in sensitivity mainly being dependent upon the work-up procedure used but also on the criteria used to evaluate the sensitivity.

The sensitivity of the fluorometric detector after pre- [207] or post- [188] column oxidation of the purines is about 2 ng/ml.

4. VINCA ALKALOIDS

The vinca alkaloids, vinblastine (Velbe), vincristine (Oncovin) and vindesine, have been employed for many years as chemotherapeutic agents in the treatment of various neoplastic diseases, especially the leukaemias and lymphomas. In spite of their frequent use, little is known about their disposition, mainly due to the lack of sensitive and selective assay procedures. Almost nothing is known of the metabolic pattern of the vinca alkaloids and the influence of the metabolites on the therapeutic efficiency in man. A study of the clinical pharmacokinetics of vinblastine during continuous intravenous infusion in man [214], using chromatographic and radiochemical techniques, revealed considerable amounts of metabolites in the plasma. Deacetylvinblastine has

been identified as the main metabolite of vinblastine [215]. This compound is more biologically active than the parent drug.

Methods for the determination of vinca alkaloids include RIA [216—220], biological methods using flow cytofluorometry [221, 222] and enzyme-linked immunosorbent assay (ELISA) [223]. Though highly desirable, chromatographic procedures are not available at the moment.

4.1. Non-chromatographic procedures

4.1.1. Radioimmunoassay

RIA procedures for the determination of vinca alkaloids in biological samples have a sensitivity of 2—5 ng/ml [216—220]. The main drawback of the methods is the long incubation times required and the lack of selectivity. Only little information is given concerning accuracy and precision.

4.1.2. Flow cytofluorometry

The biological methods using flow cytofluorometry for the determination of vinca alkaloids in blood plasma and tumour tissue have a sensitivity of 5 ng/ml [221, 222]. The methods are based on the miotic inhibitory activity of the vinca alkaloids. The methods are time-consuming, requiring 8—12 h of incubation. The selectivity of the methods is low.

4.1.3. Enzyme-linked immunosorbent assay

The sensitivity of the ELISA procedure for the determination of vinblastine and vincristine in plasma [223] is 1 ng/ml using 50-μl samples. The selectivity of the procedure and the time for analysis are comparable with those of the RIA procedures.

5. ANTIBIOTICS

5.1. Mitomycin

Mitomycin is an antitumour antibiotic, discovered in the late 1950s. Early clinical trials revealed severe toxicity, and the importance of proper dosage schedules to reduce toxicity has been emphasized.

The mechanism of action of mitomycin has been extensively studied in model systems. In its natural state mitomycin is unreactive. Mitomycin behaves as a bifunctional "alkylating" agent upon chemical or enzymatic reduction of the benzoquinone ring. Its active form is the semiquinone radical rather than the completely reduced hydroquinone (Fig. 2). In this form both the aziridine ring and the carbamate groups are able to form covalent linkage with guanine.

All analytical procedures describe the determination of the intact mitomycin only.

5.1.1. Non-chromatographic procedures

5.1.1.1. Polarography. A differential pulse polarographic method for the determination of mitomycin in plasma and urine has been presented [224]. The limit of determination (2-ml sample) was 25 ng/ml, using column extraction as a purification step.

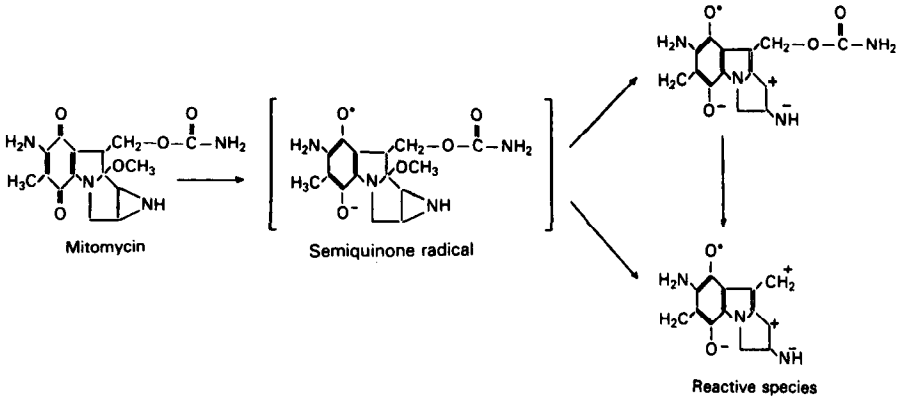

Fig. 2. Metabolic conversion of mitomycin into reactive species.

5.1.1.2. Enzyme immunoassay. The EIA for the quantification of mitomycin (225) in plasma used β-galactosidase as a label. The sensitivity of the analytical procedure was 20 ng/ml using 50-μl samples. The procedure is time-consuming, requiring 11 h of incubation.

5.1.2. Liquid column chromatography

Mitomycin can be chromatographically isolated on both straight- and reversed-phase columns, but for bioanalysis reversed-phase LC is preferred [226].

The chemical stability of mitomycin has been extensively studied [227, 228]. The drug is rapidly decomposed under acidic and alkaline conditions, which must be taken into consideration during the handling of the samples and the analytical procedure, e.g. during extraction and chromatography.

The procedures for the determination of mitomycin in samples of biological origin differ mainly in the work-up procedures. Liquid—liquid extraction of plasma and urine samples [229—231] seems less suitable since large amounts of organic extractant are necessary because of the low partition coefficient of mitomycin [230]. Alternatively, liquid—solid extraction can be used, which simplifies the work-up procedure [224, 226, 228, 232]. During the subsequent evaporation step the temperature should not exceed 40°C to avoid decomposition of the drug [230]. Den Hartigh et al. [230] found that the dissolution of mitomycin from evaporated plasma extracts using a mobile phase containing 25% of methanol was neither complete nor reproducible, which necessitated the use of pure methanol for dissolution of the drug, which might give rise to disturbances of the chromatographic behaviour. The addition of desipramine simplified the dissolution [228].

Mitomycin was isolated from coextracted compounds by reversed-phase isocratic LC using methanol [228—230] or acetonitrile [226, 227] as organic modifier. The use of a gradient system, as has been used in ref. 232, gives no advantages over the use of isocratic systems for the determination of intact drug only, but may shorten the time of analysis for HPLC and MS analysis of mitomycin, mitosane and mitosene derivatives [233].

The sensitivity of the methods using photometric detection at 360—365 nm was 2—5 ng/ml, using 1-ml plasma samples, the yield being approximately 80%.

The use of porfiromycin as internal standard [231, 232] gives no advantage over external standardization [226, 228—230] in respect of accuracy. The electrochemical detection of mitomycin [226] was less favourable than photometric detection, being somewhat less sensitive.

5.2. Anthraquinone glycosides

Anthraquinone glycosides have been used for the treatment of leukaemia and solid tumours for about twenty years. Adriamycin and daunorubicin are the most frequently used drugs within this class. Adriamycin and daunorubicin are extensively metabolized (Fig. 3), but only the 14-hydroxy derivatives are of clinical importance; in model systems they have been reported to have about the same cytotoxic activity as the intact drugs. From the clinical point of view it is of a great importance to measure plasma levels not only of the intact drugs, but also of their reduced metabolites, since a high therapeutic response has been suggested to be associated with a high degree of formation of reduced metabolites [234].

To avoid erroneous results when determining anthraquinone glycosides in blood and plasma, special precautions have to be included in the sampling procedures [235].

The stability of the anthraquinone glycosides is strongly dependent upon pH. The degradation rate increases with increasing pH. Phosphoric acid is recommended as solvent for stock solutions [236].

Adriamycin and daunorubicin are enzymatically reduced in whole blood to adriamycinol and daunorubicinol, respectively. It is essential to separate the plasma fractions immediately after collection of the blood to obtain correct information of the relative amounts of intact drug and reduced metabolite [235].

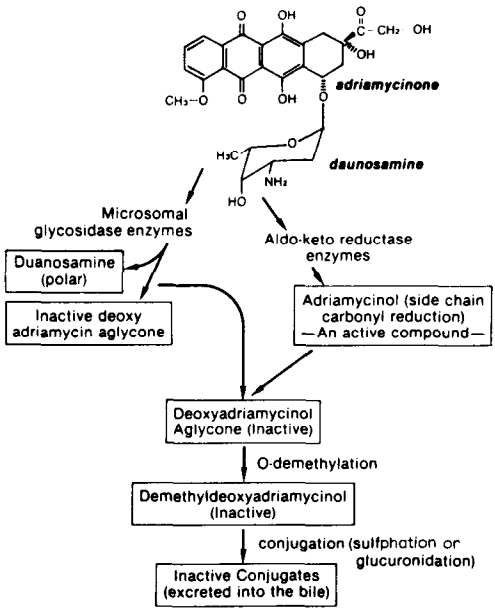

Fig. 3. Metabolism of adriamycin.

Studies on stability in plasma containing adriamycin and daunorubicin [235] showed a drastic decrease of the anthraquinone glycoside concentration after repeated freezing and thawing as well as after storage of spiked plasma samples at −20°C. This effect was not due to degradation of the compounds, but was an effect originating from changes in the plasma itself. However, Oosterbaan et al. [237] observed no losses of drug under similar conditions.

The plasma half-life of adriamycin in the α-phase is about 4 min. Calculations [236] revealed that a high precision in the sampling time is necessary to obtain a high accuracy in the calculation of pharmacokinetic parameters. A precision in sampling time of ± 1 min gives reported plasma concentrations within ± 10% of the correct plasma concentration ⩽ 30 min post injection.

5.2.1. Non-chromatographic procedures

5.2.1.1. Fluorometry. Initially, the anthraquinones were quantified by measurement of total fluorescence, i.e. the total amount of the anthraquinone glycosides and aglycones was measured. Plasma and urine samples (1 ml) were initially extracted with *n*-butanol [238, 239]. The total yield was about 80% with a sensitivity of 50 ng/ml. The anthraquinone content in tissue was determined in a similar way after extraction with 0.3 *M* hydrochloric acid—50% ethanol [240], with a yield > 98%. This method, however, suffered from high blank values, and was subsequently improved by Schwartz [241], who released drugs in bound form by treatment with Ag(I) before the butanol extraction.

Most importantly, the fluorescent properties of the anthraquinones have been used for quantification within single cells by cytofluorescence, with either photomicrographic [242—244], or flow cytometric techniques using a high-pressure xenon lamp [245] or a laser [246—249] as the excitation source.

5.2.1.2. Electrochemical technique. The electrochemical reduction of adriamycin has been reported in several polarographic studies at mercury [250—252] and carbon paste [253] electrodes. At negative potentials two sets of reduction waves were observed, corresponding to the reduction of the quinone and the carbonyl side-chain, respectively.

The use of differential pulse polarography for the determination of total anthraquinones in blood plasma [252], using the quinone reduction current, gives a sensitivity of 400 ng/ml with a precision of ± 5%. The sensitivity of this method is apparently not sufficient for pharmacokinetic studies. No sample manipulation or preparation is required, giving a total analysis time of less than 5 minutes.

Anthraquinones in urine have been determined electrochemically by preconcentration at carbon paste electrodes [254]. Although somewhat higher detection sensitivity was obtained compared to [252], the two methods suffer from lack of selectivity as not only anthraquinone glycosides are determined but also their aglycones.

5.2.1.3. Radioimmunoassay. Two procedures for the determination of anthraquinone glycosides using RIA are described in the literature [255, 256]. Both procedures have a very high sensitivity, permitting quantitative determinations in the low ng/ml range of the drugs in plasma.

The ^{125}I RIA for adriamycin [255], now commercially available, gave values five- to ten-fold those obtained by ^{3}H RIA [256]. The RIA technique is

less suitable for quantitative determinations of anthraquinone glycosides in plasma samples since not only are intact drugs determined but also their metabolites. The adriamycin aglycone reacts about 25% as effectively as the intact drug [258], the reactivity of other aglycones varies between 7% and 80% [257].

RIA and the fluorometric technique for the determination of adriamycin and daunorubicin gave similar results 0—4 h after drug administration. From 4 through 72 h after drug administration the fluorescence assay consistently yielded higher values than RIA [258].

The low selectivity of the RIA methods has been established by comparison of RIA with a method based on HPLC [257].

5.2.1.4. Enzyme immunoassay. Recently, methods for the determination of adriamycin and daunorubicin using EIA have been developed [259, 260]. This technique offers a high sensitivity (0.5 ng/ml).

The selectivity of EIA methods for the determination of anthraquinone glycosides has not generally been evaluated. The correlation of the EIA and fluorometric methods, investigated by the assay of adriamycin in serum (0.35—18 μg/ml), was good [260].

5.2.2. Thin-layer chromatography

The metabolic pattern of adriamycin and daunorubicin was studied by TLC ([261, 262] and refs. therein), but the systems used were not designed for quantitative purposes. Risk of formation of artefacts due to the unsuitable extraction systems used [263] limited the value of the quantitative information given.

Modern methods for the quantitative determination of adriamycin and its metabolites in biological tissues and plasma by TLC use a thin-film fluorescence scanner for the quantitative evaluation [264, 265].

Chan and Harris [264] extracted adriamycin and its metabolites with a mixture of concentrated hydrochloric acid and ethanol. The detection limit of the method was 20 ng/ml. However, adriamycin is partly hydrolysed to the aglycone thus giving too low measurements of adriamycin concentration [265].

For the determination of adriamycin in plasma [265], daunorubicin was initially added as an internal standard. The sample was extracted with ice-cold chloroform—isopropanol (1:1). After evaporation of the extract and dissolving in 3—4 μl of chloroform—ethanol (1:1), the entire sample was spotted on a silica gel plate, followed by two-dimensional TLC. The excitation wavelength of the thin-film fluorescence scanner was set at 475 nm and the emission at 580 nm. The detection limit was < 1 ng/ml, obtained with spiked plasma, the precision being $\leqslant 4\%$.

One alternative to the TLC method given in ref. 265 is that presented by Gessner et al. [266], who used chloroform—isopropanol (1:1) saturated with ammonium sulphate as the extraction medium. After TLC separation the area on the TLC plates containing adriamycin was scraped off and the silica gel was eluted with hydrochloric acid in methanol. The fluorescence was read on a spectrofluorometer (470 nm/585 nm).

5.2.3. Liquid column chromatography

The most convenient and accurate methods for the determination of anthraquinone glycosides are based on liquid column chromatography. The majority of published methods for the determination of anthracyclines deal with daunorubicin and adriamycin, but these methods can easily be adopted for almost all compounds within this class. Methods for the determination of newer anthracyclines include those for aclacinomycin [267—270], carminomycin [237, 268, 271, 272], marcellomycin [268], rubidazon [273, 274], 4'-epiadriamycin [237, 275—277], deoxyadriamycin [237], demethoxyadriamycin [237], N-trifluoroacetyladriamycin (AD32) [278] and demethoxydaunorubicin [237, 279].

The work-up procedures for the determination of anthraquinone glycosides in biological samples include liquid—liquid extraction [237, 267, 268, 276, 277, 280—291], liquid—solid extraction [270, 292, 293], precipitation of plasma proteins [281, 294, 295] and direct injection (urine samples) [296].

The extraction properties of adriamycin, daunorubicin and their reduced metabolites in liquid—liquid systems were studied by Eksborg [297]. The degree of extraction varied with pH of the aqueous phase, with optimal degree of extraction at pH 8.0 (daunorubicin), 8.1 (daunorubicinol), 8.4 (adriamycin) and 8.6 (adriamycinol). The degree of extraction decreased with increasing concentration due to the formation of dimers and tetramers. Too high a pH should be avoided because of the risk of chemical degradation of the anthraquinone glycosides [236], and it is doubtful that a pH value as high as 9.8 [285] is useful.

Liquid—solid extraction of the anthraquinone glycosides from biological fluids for HPLC evaluation is an attractive alternative to liquid—liquid extraction as the initial step of the analytical procedure [298]. Compared to liquid—liquid extraction the use of C_{18} Sep-Pak extraction columns is reported to have the following advantages: speed, simplicity, efficacy, reproducibility and similar recoveries of various anthracyclines [298]. However, neither yield, accuracy nor precision data are presented.

It was early noted that considerable losses could occur due to adsorption to glass surfaces during the extraction procedure. This was prevented by the addition of a secondary amine, desipramine, to the samples [284, 286].

Mostly, the initial extract containing the anthraquinone glycosides is evaporated to dryness, followed by redissolution in mobile phase or in the mobile phase modifier prior to injection into the chromatographic system [267, 268, 271, 272, 277, 280, 283, 287, 288]. Although this technique allows direct determination of the intact drug, reduced metabolite and aglycones, the risk of losses due to adsorption should be taken into consideration.

For the determination of intact drug and corresponding active metabolite, the 14-hydroxyanthraquinone glycoside, a reextraction step should preferably be included [270, 276, 279, 284, 286, 289]. From data presented in ref. 297, it can be concluded that a weakly acidic aqueous phase such as dilute phosphoric acid is suitable for this purpose.

Precipitation of proteins is a commonly used work-up procedure for the analysis of drugs and metabolites in plasma samples. It is a less suitable technique for the determination of low concentrations of anthraquinone

glycosides in plasma because of the high risk of loss of drugs and metabolites by coprecipitation, (cf. ref. 236). This drawback can to some extent be compensated for by the use of internal standards prior to the addition of the precipitation reagent.

Liquid—solid extraction on-line [270] is an attractive alternative to liquid—liquid extraction of anthraquinone glycosides since it is less time consuming.

Excellent separation of intact anthraquinone drugs and corresponding reduced metabolites can be obtained by both straight- and reversed-phase liquid chromatography. Depending upon the work-up procedure used, aglycones and conjugates can also be simultaneously determined in biological samples by some of the methods [268, 281, 283, 285, 287, 288, 292, 295], as well as degradation products for the analysis of pharmaceutical preparations [299, 300].

Because of the large batch-to-batch variation of the support materials no definite conclusion concerning the most suitable material for the separation of anthracyclines can be drawn. For the separation of the intact drug and corresponding reduced metabolite a fairly simple isocratic chromatographic system can be used. Isocratic chromatographic systems can also be used for determinations including aglycones [268, 285, 287, 292, 295, 301] (cf. refs. 281 and 283). Gradient systems are necessary only for complete characterization of intact drugs and metabolites, i.e. analysis including intact drugs, 14-hydroxy metabolites, aglycones, O-glucuronides and O-sulphates [277, 288].

A study of the separation of adriamycin and daunorubicin, adriamycinol and daunorubicinol, on LiChrosorb RP-2, RP-8 and RP-18 [301] states that the selectivity of the chromatographic systems increased with decreasing organic modifier in mobile phase, consisting of 10^{-2} *M* phosphoric acid. Acetonitrile was superior to alcohols for the separation of intact drug from the respective reduced metabolite. The selectivity using alcohols as modifier decreased in the order methanol, ethanol, isopropanol. Addition of sodium dodecyl sulphate to the mobile phase has been reported to have a beneficial effect on the general performance of the chromatographic systems [302].

Fluorometric detection is the most commonly used technique for quantification of anthracyclines in column eluate. Photometric detection at 500 nm gives almost the same sensitivity and selectivity as the fluorometric detectors. Neither the combination of HPLC with RIA nor electrochemical detection of anthracyclines offers any advantages with respect to sensitivity compared to fluorometric and photometric detectors.

In general, only scant information is presented concerning sensitivity, precision and accuracy in the methods published. The practical limit for quantitative determination of anthracyclines in biological samples can approximately be estimated to 2—5 ng/ml when using 1 ml of plasma or urine.

5.2.4. Mass spectrometry

EI-MS is a very useful tool for the structural determination of anthracyclinones [303] and amino sugars [304], but it is not applicable to the parent compounds because of their thermal lability and low volatility. Derivatization does not improve the stability sufficiently. However, peracetylation of some

daunorubicin derivatives has been described [305] as well as pertrimethylsilylation [306, 307] (for aglycones only). A soft ionization technique is needed to obtain accurate mass spectra from underivatized compounds. FD-MS was used to examine adriamycin and daunorubicin [308] and newer anthracycline derivatives [309]. Counter-current chromatography is recommended for the separation process prior to MS analysis [310] as an alternative to HPLC to avoid disturbances from support materials.

5.2.5. Isotachophoresis

Isotachophoretic determination of adriamycin and adriamycinol in human plasma is described in ref. 311. The plasma is extracted with *n*-butanol. The extracts are evaporated and redissolved in methanol before being subjected to isotachophoresis. The sensitivity of the method is about 5 ng/ml.

5.3. Aminoanthraquinones

Aminoanthraquinones, bis(substituted aminoalkylamino)anthraquinones, are a new class of cancer chemotherapeutic agents developed as a result of structure—activity studies among DNA intercalating agents. These types of compounds have very high activity against murine tumours including P388 and L1210 leukaemias, B16 melanoma and colon carcinoma 26. Ametrantone (NSC-287513), bisantrene (NSC-337766) and mitoxantrone (NSC-301739) are the most promising new drugs within this class of compounds.

The aminoanthraquinones are unstable under normal physiological conditions and special precautions need to be taken to avoid erroneous results when analysing them. Mitoxantrone is less stable in human plasma than bisantrene [312]. The degradation probably involves oxidation of the phenylenediamine moiety to the corresponding quinoneimine which is subject to hydrolysis to yield the quinone. Such reactions are pH-dependent, and the stability can be enhanced by decreasing the pH [313]. Ascorbic acid proved to be a useful stabilizing agent, but because of its instability at neutral pH, acidification of plasma samples concurrent with the addition of ascorbate was necessary [312, 313]. Exposure to fluorescent room light promotes degradation of bisantrene [314].

5.3.1. Liquid column chromatography

All methods available for the determination of aminoanthraquinones are based on HPLC. A systematic study of the chromatographic properties of these compounds [315] states that straight-phase chromatography with solvent combinations of chloroform with various alcohols is unsuitable because of highly retained, broad and/or skewed peaks. Poor resolution of the aminoanthraquinones was found on μBondapak C_{18} columns using aqueous mixtures of methanol or acetonitrile as mobile phase. The μBondapak CN and μBondapak NH_2 columns proved suitable for the analysis of aminoanthraquinones. The column performance was further increased by replacement of water in the mobile phase with various salt solutions. Buffers containing formate ions were superior to those containing phosphate and acetate ions, since a considerable reduction of the peak tailing was observed [316]. The

column performance could also be enhanced by an increase in column temperature (50°C) [313, 315], as well as the addition of ion-pairing agents to the mobile phase [313, 317].

Work-up procedures for the determination of aminoanthraquinones in biological fluids (plasma, urine, bile, etc.) include liquid—liquid extraction [314, 316—318] and liquid—solid extraction, using either a Sep-Pak C_{18} cartridge (312, 319, 320] or a XAD-2 column (313]. The use of Sep-Pak extraction columns was faster and more accurate than the other extraction techniques used [312].

The eluate from the chromatographic columns was monitored by photometric [312—314, 316—318], fluorometric [320] and electrochemical [319] detection, with internal [314, 316, 317] or external [312, 313, 318, 320] standardization. The sensitivity for mitoxantrone analysis was typically 1 ng/ml [312, 316, 317] with photometric detection at 546, 605 and 436 nm, respectively. A detection limit of 75 ng/ml was found using a less selective detection at 254 nm [313]. Photometric detection of bisantrene [314, 318] had a detection limit of 25 ng/ml, which could be increased by using fluorometric [320] or electrochemical detection [319].

5.4. Amsacrine

Amsacrine, 4′-(9-acridinylamino)metanesulfon-*m*-anisidide, is an acridine derivative currently undergoing clinical evaluation as a chemotherapeutic agent for the treatment of human tumours. The drug has demonstrated significant activity against leukaemias and lymphomas, and has moderate activity against metastatic breast cancer and malignant melanoma. The metabolism of amsacrine is not known.

5.4.1. Non-chromatographic procedures

5.4.1.1. Fluorometry. The conversion of amsacrine under alkaline conditions to highly fluorescent acridone has been used for the determination of the drug in plasma [321]. The analytical procedure comprises an extraction step with benzene followed by evaporation of the extract, treatment with sodium hydroxide solution and fluorometric quantification (266 nm/470 nm). The method is linear within the range 4—400 ng/ml.

5.4.2. Gas chromatography

The GC method for the determination of amsacrine [322] comprises extraction into chloroform, purification of the extract by solvent extraction and isolation of intact amsacrine by GC with FID or NPD. The detection limit of the method is approximately 50 ng/ml (NPD) and 125 ng/ml (FID).

5.4.3. Liquid column chromatography

Two procedures for the determination of amsacrine in plasma based on reversed-phase LC are described [323, 324]. In both methods the clean-up procedure is initiated by removal of lipid materials by acidification and extraction with hexane, followed by alkalinization and transfer of amsacrine to an organic phase.

Reversed-phase chromatography with methanol [323] or acetonitrile—triethylamine phosphate [324] as modifier in the mobile phase was used. The sensitivity was about 20 ng/ml.

5.5. Bleomycin

The antitumour antibiotic bleomycin is approved for the chemotherapy of squamous cell carcinoma, lymphoma and testicular carcinoma, although antitumour activity has been noted for other malignancies. Bleomycin is a mixture of at least thirteen closely related, water-soluble glycopeptides, which differ only in their terminal amines. The A_2 and B_2 fractions make up at least 80% of the total weight of the commercially available preparations. Minor components include A_1, A_5, B_1, B_4, demethyl-A_2 and bleomycinic acid. The potency of the bleomycin mixtures is in general determined by microbiological assay, even though the antibacterial activity of the different bleomycins does not necessarily parallel their antitumour activity.

The commercially available preparations have been characterized by means of chromatographic methods [325—330]. The initial attempts to separate the constituents of bleomycin by column chromatography resulted in time-consuming analysis, with typical separation times > 4 h [325, 328, 329]. The use of modern, reversed-phase chromatography decreased the separation time considerably [326, 327], but the efficiency of the chromatographic systems was low, with strongly tailing peaks [327]. Recently, an Ultrasphere ion-pair column was used for the separation of bleomycin components [330]. The use of gradient elution with a mobile phase composed of acetonitrile—0.01 *M* sodium citrate buffer pH 4—0.02 *M* hexane sulphonic acid sodium salt—0.005 *M* disodium ethylenediaminetetraacetate resulted in very efficient separations.

Bleomycin undergoes significant metabolic conversion, as evident from a 45% recovery of unchanged drug in treated cancer patients. However, the structure and the importance of the metabolites for the therapeutic efficiency and side-effects are unknown.

Bleomycin is very sensitive to photolysis and on irradiation it undergoes a number of photoinduced reactions [331]. The importance of the photoprocesses for the handling of samples of biological origin prior to analysis has not yet been evaluated.

Three different techniques have been used for the determination of bleomycin in biological samples, namely RIA [332—335], enzyme inhibition assay [336, 337] and LC [338, 339].

5.5.1. Non-chromatographic procedures

5.5.1.1. Radioimmunoassay. The RIA methods for the determination of bleomycin in biological samples utilize ^{125}I-labelled [332] or ^{57}Co-labelled [334] bleomycin as tracers. The sensitivity of the procedures is 25 ng/ml with a precision of 2.8%.

The immunoreactivity of bleomycin analogues relative to bleomycin sulphate have been systematically studied [334]. Bleomycin analogues that were in the mixture cross-reacted with ^{125}I-labelled bleomycin sulphate (A_2, B_2, A_5) as did isobleomycin A_2 and desamidobleomycin. Bleomycinic acid did not

significantly compete for antibody binding. Bleomycin B_1 competed with low efficiency.

An RIA for the determination of pepleomycin, an analogue of bleomycin, distinguished from bleomycin by substitution in the terminal amine position, did not cross-react with bleomycin [335].

5.5.1.2. Enzyme inhibition assay. The enzyme inhibition assays for the determination of bleomycin [337] and pepleomycin [336] are considerably more sensitive than RIA methods, with a limit of detection of about 0.5 ng/ml. The specificity for the antisera was carefully evaluated. No cross-reactivity between bleomycin and pepleomycin occurred.

5.5.2. Liquid column chromatography

Bleomycin A_2 has been determined in plasma and urine by reversed-phase HPLC with photometric detection at 254 nm [338, 339]. A μBondapak C_{18} column was used with a mobile phase of heptane sulphonic acid in methanol-diluted acetic acid. The plasma samples were injected after precipitation of plasma proteins with trichloroacetic acid. A liquid—solid extraction procedure with the aid of Sep-Pak columns was used for the determination of bleomycin in urine samples. The sensitivity was 500 ng/ml of plasma and 5 μg/ml of urine. The chromatographic system used permitted separation of bleomycin A_1, A_2 and B_2, but showed a very low separation efficiency and tailing peaks.

6. *cis*-DIAMMINEDICHLOROPLATINUM(II)

cis-Diamminedichloroplatinum(II) (CDDP) has proven efficacy in the treatment of germinal neoplasma of the testis, advanced ovarian carcinoma, and head and neck cancer.

The reactivity of CDDP in plasma with special reference to the handling of plasma samples for pharmacokinetic studies is given in ref. 340. CDDP was found to be unstable in urine, and could only be successfully kept without significant degradation by storage over solid carbon dioxide (ca. —60°C) [341].

6.1. Non-chromatographic procedures

6.1.1. Atomic absorption, X-ray fluorescence and polarographic assays

Early analytical methods for the determination of CDDP monitor the total platinum in the biological samples by use of flameless atomic absorption spectrophotometry [342—350]. They were, however, not able to discriminate between free and protein-bound platinum species nor were they capable of differentiating among the various platinum species that may be present. As it is assumed that the drug can only exert chemotherapeutic action in the free form, the ability to discriminate between this state and the bound form is essential for meaningful drug concentration evaluation.

Protein-bound and free platinum species have been separated by centrifugal ultrafiltration followed by conversion of the unbound fraction of CDDP to a stable cation species by reaction with ethylenediamine. The product was collected on a paper disc impregnated with cation resin by filtration followed by quantification by X-ray fluorescence spectroscopy [351] or flameless atomic

absorption [352, 353]. Alternatively, an excess of ammonium 1-pyrrilidine-dithiocarbamate was added to the ultrafiltrate and the formed platinum complex was extracted into isopropylacetone prior to measurement by flameless atomic absorption [354].

To overcome the variability in the platinum absorption signal attributed to the composition of the sample matrix, extensive sample preparation has been employed when utilizing atomic absorption spectroscopy. Both wet [342—347, 349, 354] and dry [355] digestion procedures have been used. These harsh techniques are often inaccurate, and could be eliminated by careful optimization of instrument parameters [350].

Atomic absorption spectrophotometry has a detection limit for platinum of about 10—40 ng/ml. Polarographic assay of platinum offers a considerable increase in detection sensitivity, the limit of detection being 0.5 ng/ml [356, 357].

6.2. Liquid column chromatography

The liquid column chromatographic properties of CDDP and related platinum-containing compounds have been extensively studied by Sternson and co-workers [358—365]. The use of solvent-generated anion exchangers for the analysis of CDDP offers significant advantages over the chemically bonded systems in terms of peak shape, column efficiency, and stability. Solvent-generated anion-exchange HPLC was applied to the analysis of CDDP in urine by automated column switching [361]. On-line electrochemical detection of the HPLC eluent in both oxidative and reductive modes provides a significant advantage over UV absorbance and off-line flameless atomic absorption detectors [365, 366].

Isolation of CDDP in plasma ultrafiltrate on a strong cation-exchange column gives a sensitivity of 1 μg/ml using 7 ml of plasma. Using off-line atomic absorption spectrophotometric determination of the eluate a sensitivity of 40 ng/ml was obtained [363, 364].

Complexation of CDDP with diethyldithiocarbamate with straight- [365—367] or reversed-phase [368] isolation of the complex and photometric determination gives a sensitivity of 25 ng/ml of urine or plasma ultrafiltrate. Palladium chloride [367] or nickel(II) [366] was used as the internal standard while external standardization was used in other methods [365, 368]. In conclusion, the methods involving precolumn derivatization with diethyldithiocarbamate provide the required sensitivity but suffer from lack of selectivity as they only respond to total platinum levels.

Liquid column chromatographic separation of the underivatized compounds [358, 359] preceding the derivatization step has been suggested to enhance detection sensitivity [361, 369]. A post-column reaction detector sensitive to platinum(II) complexes has been described [369] in which sodium bisulphite is used as the derivatizing agent with potassium dichromate as an activating agent. The detection limit for CDDP was 40 ng/ml.

The stability of CDDP in aqueous parenteral vehicles was studied by a dual-column system [370] consisting of both a strong cation column and a strong anion column connected in series, permitting separation of intact drug from degradation products.

7. PODOPHYLLOTOXIN DERIVATIVES

Etoposide (VP 16-213) and teniposide (VM 26), semisynthetic derivatives of podophyllotoxin, are promising new antineoplastic agents, active against small-cell anaplastic bronchial carcinoma, larger-cell bronchial carcinoma, acute and chronic leukaemias, lymphomas, testicular teratomas and central nervous system malignancies.

7.1. Liquid column chromatography

All methods available for the determination of non-radioactive etoposide and teniposide in biological samples (plasma, urine, liquor) are based on reversed-phase liquid chromatography [371—377].

Procedures for the initial isolation of the drugs are based on liquid—liquid extraction, using chloroform or 1,2-dichloroethane as extractant, evaporation of the organic extract, and subsequent redissolution in mobile phase (methanol—buffer). The chromatographic separation from coextracted endogenous materials is made on a reversed-phase column (phenyl, C_8 or C_{18}), with photometric [371—373, 375), fluorometric [371, 374, 376] or electrochemical [377] detection. The analytical procedure using the electrochemical detection system was the most sensitive, with a detection limit of 2 ng/ml, while the sensitivity of the photometric and fluorometric detection systems was 30—50 ng/ml.

A fully automated HPLC system for the analysis of etoposide and teniposide has also been described [376]. The blood samples are hydrolysed by an enzyme, subtilisin A, followed by preconcentration on a small precolumn on-line. Chromatography takes place after column switching in a C_{18}/methanol—water system. After a post-column clean-up step on-line, native fluorescence of the analytes is used for quantification, the limit of detection being 10 ng/ml.

The metabolism of etoposide and teniposide is poorly understood, although the compounds have been extensively studied in clinical trials and in vivo. By the use of a phenyl reversed-phase column it was possible to resolve etoposide, its *cis*-isomer and the *cis*-hydroxy acid of the aglycone under optimal chromatographic conditions [377], but only intact etoposide was detected in patients' plasma.

8. NITROSOUREAS

The most commonly used nitosureas are BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea], CCNU [1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea] and MeCCNU [1-(2-chloroethyl)-3-(4-*trans*-methylcyclohexyl)-1-nitrosourea]. Their chemical properties allow them to penetrate the blood—brain barrier and they are thus useful in the treatment of tumours in the central nervous system. The pharmacological effect originates from their rapid non-enzymatic decomposition to unstable intermediates capable of DNA alkylation.

The nitrosoureas rapidly decompose at physiological pH and macromolecular components have been found to increase the rate of degradation in plasma

[378]. The mechanism of hydrolysis of the nitrosoureas has been thoroughly studied [379] and the highest stability is observed in the pH range 2—4.

8.1. Gas chromatography

GC with SIM has been utilized for the determination of BCNU, CCNU, and MeCCNU in plasma [380]. The method is based on conversion of the nitrosoureas to the corresponding 1,3-diacyl-1,3-dialkylureas by reaction with trifluoroacetic anhydride. Since denitrosated metabolites give the same derivatives as the parent compounds it is necessary to separate the nitrosoureas from any denitrosated metabolites by TLC prior to the derivatization step. The limit of sensitivity in plasma is 1—3 ng/ml.

8.2. Liquid column chromatography

Reversed-phase LC with photometric detection at 230—237 nm has been used for the determination of BCNU [381, 382] and MeCCNU [383]. Yeager et al. [381] stabilized BCNU in plasma by adjusting the pH to 4 before isolating BCNU by solvent extraction. The limit of sensitivity of the methods is in the range 30—100 ng/ml.

8.3. Mass spectrometry

Direct-inlet MS using CI has been used for the determination of BCNU in biological fluids [384]. Since BCNU evaporates readily from the insertion probe the masses of interest can be detected without significant interference from other sample constituents. The limit of sensitivity is about 80 ng/ml of plasma.

9. CONCLUSIONS

The increasing importance and use of anticancer drugs for the treatment of neoplastic diseases has stressed the importance of individualized therapy based on measured plasma, urine or tissue levels of the drugs used. Procedures for the determination of cytostatics in biological samples should include a chromatographic step to ensure the required selectivity. It is desirable that not only the intact drugs but also metabolites with pharmacological activity are determined.

The data obtained on the concentrations of drugs and metabolites should be interpreted with care. No simple correlations between plasma concentrations and therapeutic efficacy can be expected, since most of the drugs exhibit their cytostatic activity by binding irreversibly within the tumour cells. At the present time the most potential use of pharmacokinetic data for anticancer drugs is to reduce side-effects, e.g. by comparative studies of the pharmacokinetics after intravenous and locoregional administration.

For a vast number of anticancer drugs the metabolism, mode of action and pharmacokinetics are still unknown, due to the lack of suitable analytical procedures. Our knowledge, however, will be increased with the increasing use of analytical techniques with high selectivity and sensitivity.

10. SUMMARY

The present review on the quantification of cytostatic drugs has mainly been focussed on chromatographic techniques. Special attention has been paid to the precautions that have to be taken into account to ensure the selectivity and accuracy of the various methods.

The various cytostatics that have been dealt with are: alkylating agents, antimetabolites, vinca alkaloids, antibiotics, *cis*-diamminedichloroplatinum, podophyllotoxine derivatives, and nitrosoureas.

REFERENCES

1 A. Aszalos, J. Liquid Chromatogr., 7 (Suppl. 1) (1984) 69—125.
2 A. McLean, R.L. Woods, D. Catovsky and P. Farmer, Cancer Treat. Rev., 6 (Suppl.) (1979) 33—42.
3 D.S. Alberts, S.Y. Chang, H.-S.G. Chen, B.J. Larcom and S.E. Jones, Cancer Treat. Rev., 6 (Suppl.) (1979) 9—17.
4 D.R. Newell, A.H. Calvert, K.R. Harrap and T.J. McElwain, Brit. J. Clin. Pharmacol., 15 (1983) 253—258.
5 H. Ehrsson, I. Wallin, B. Simonsson, P. Hartvig and G. Öberg, Eur. J. Clin. Pharmacol., 27 (1984) 111—114.
6 G.E. Goodman, A. McLean, D.S. Alberts and S.Y. Chang, Brit. J. Cancer, 45 (1982) 621—623.
7 W.R. Owen and P.J. Stewart, J. Pharm. Sci., 68 (1979) 992—996.
8 H. Ehrsson, S. Eksborg, I. Wallin and S.-O. Nilsson, J. Pharm. Sci., 69 (1980) 1091—1094.
9 D.C. Chatterji, R.L. Yeager and J.F. Gallelli, J. Pharm. Sci., 71 (1982) 50—54.
10 J.H. Linford, Can. J. Biochem. Physiol., 41 (1963) 931—939.
11 H. Ehrsson, U. Lönroth, I. Wallin, M. Ehrnebo and S.O. Nilsson, J. Pharm. Pharmacol., 33 (1981) 313—315.
12 T. Jakhammer, A. Olsson and L. Svensson, Acta Pharm. Suec., 14 (1977) 485—496.
13 S.Y. Chang, B.J. Larcom, D.S. Alberts, B. Larsen, P.D. Walson and I.G. Sipes, J. Pharm. Sci., 69 (1980) 80—84.
14 H. Ehrsson, S. Eksborg, I. Wallin, Y. Mårde and B. Joansson, J. Pharm. Sci., 69 (1980) 710—712.
15 P. Leff and W.G. Bardsley, Biochem. Pharmacol., 28 (1979) 1289—1292.
16 A.E. Ahmed, M. Koenig and H.H. Farrish, Jr., J. Chromatogr., 233 (1982) 392—397.
17 D.R. Newell, L.I. Hart and K.R. Harrap, J. Chromatogr., 164 (1979) 114—119.
18 M. Zakaria and P.R. Brown, J. Chromatogr., 230 (1982) 381—389.
19 S.Y. Chang, D.S. Alberts, L.R. Melnick, P.D. Walson and S.E. Salmon, J. Pharm. Sci., 67 (1978) 679—682.
20 K.P. Flora, S.L. Smith and J.C. Cradock, J. Chromatogr., 177 (1979) 91—97.
21 S.Y. Chang, T.L. Evans and D.S. Alberts, J. Pharm. Pharmacol., 31 (1979) 853—854.
22 S.Y. Chang, T.L. Evans, D.S. Alberts and I.G. Sipes, Life Sci., 23 (1978) 1697—1702.
23 T.L. Evans, S.Y. Chang, D.S. Alberts, I.G. Sipes and K. Brendel, Cancer Chemother. Pharmacol., 8 (1982) 175—178.
24 H. Ehrsson and U. Lönroth, J. Pharm. Sci., 71 (1982) 826—827.
25 I.A.-K. Taha, R.A.-J. Ahmad and H.J. Rogers, Cancer Chemother. Pharmacol., 5 (1981) 181—184.
26 R.L. Furner, L.B. Mellett, R.K. Brown and G. Duncan, Drug Metab. Dispos., 4 (1976) 577—583.
27 A.E. Ahmed and T.-F. Hsu, J. Chromatogr., 222 (1981) 453—460.
28 A.G. Bosanquet and E.D. Gilby, J. Chromatogr., 232 (1982) 345—354.
29 C.M. Egan, C.R. Jones and M. McCluskey, J. Chromatogr., 224 (1981) 338—342.

30 M.H.N. Tattersall, M. Jarman, E.S. Newlands, L. Holyhead, R.A.V. Milstead and A. Weinberg, Eur. J. Cancer, 14 (1978) 507—513.
31 S.L. Pallante, C. Fenselau, R.G. Mennel, R.B. Brundrett, M. Appler, N.B. Rosenshein and M. Colvin, Cancer Res., 40 (1980) 2268—2272.
32 M. Hassan and H. Ehrsson, J. Pharm. Biomed. Anal., in press.
33 H. Ehrsson and M. Hassan, J. Pharm. Pharmacol., 36 (1984) 694—696.
34 H. Vodopick, H.E. Hamilton, H.L. Jackson, C.T. Peng and R.F. Sheets, J. Lab. Clin. Med., 73 (1969) 266—276.
35 H. Ehrsson and M. Hassan, J. Pharm. Sci., 72 (1983) 1203—1205.
36 M. Hassan and H. Ehrsson, J. Chromatogr., 277 (1983) 374—380.
37 L.B. Grochow and M. Colvin, Clin. Pharmacokin., 4 (1979) 380—394.
38 M. Colvin and J. Hilton, Cancer Treat. Rep., 65 (Suppl. 3) (1981) 89—95.
39 O.D. Boughton, R.D. Brown, R. Bryant, F.J. Burger and C.M. Combs, J. Pharm. Sci., 61 (1972) 97—100.
40 T. Facchinetti, M. d'Incalci, G. Martelli, L. Cantoni, G. Belvedere and M. Salmona, J. Chromatogr., 145 (1978) 315—318.
41 E.A. de Bruin, U.R. Tjaden, A.T. van Oosterom, P. Leeflang and P.A. Leclercq, J. Chromatogr., 279 (1983) 603—608.
42 N. van den Bosch and D. de Vos, J. Chromatogr., 183 (1980) 49—56.
43 C. Pantarotto, A. Martini, G. Belvedere, M.G. Donelli and A. Frigerio, Cancer Treat. Rep., 60 (1976) 493—500.
44 B. Whiting, S.H.K. Miller and B. Caddy, Brit. J. Clin. Pharmacol., 6 (1978) 373—376.
45 F.D. Juma, H.J. Rogers, J.R. Trounce and I.D. Bradbrook, Cancer Chemother. Pharmacol., 1 (1978) 229—231.
46 I. Jardine, C. Fenselau, M. Appler, M.-N. Kan, R.B. Brundrett and M. Colvin, Cancer Res., 38 (1978) 408—415.
47 F.D. Juma, H.J. Rogers and J.R. Trounce, Brit. J. Clin. Pharmacol., 10 (1980) 327—335.
48 M.S.B. Nayar, L.-Y. Lin, S.H. Wan and K.K. Chan, Anal. Lett., 12 (1979) 905—915.
49 C. Pantarotto, A. Bossi, G. Belvedere, A. Martini, M.G. Donelli and A. Frigerio, J. Pharm. Sci., 63 (1974) 1554—1558.
50 T.T. Kensler, R.J. Behme and D. Brooke, J. Pharm. Sci., 68 (1979) 172—174.
51 M. Jarman, E.D. Gilby, A.B. Foster and P.K. Bondy, Clin. Chim. Acta, 58 (1975) 61—69.
52 M. Jarman, R.A.V. Milsted, J.F. Smyth, R.W. Kinas, K. Pankiewicz and W.J. Stec, Cancer Res., 39 (1979) 2762—2767.
53 P.J. Cox, P.B. Farmer, A.B. Foster, L.J. Griggs, M. Jarman, R. Kinas, K. Pankiewicz and W.J. Stec, Biomed. Mass Spectrom., 4 (1977) 371—375.
54 P.J. Cox, P.B. Farmer, M. Jarman, R.W. Kinas and W.J. Stec, Drug Metab. Dispos., 6 (1978) 617—622.
55 U. Bahr, H.-R. Schulten, O.R. Hommes and F. Aerts, Clin. Chim. Acta, 103 (1980) 183—192.
56 G. Lunglmayr and K. Czech, J. Urol., 106 (1971) 72—74.
57 K. Czech and G. Lunglmayr, II. Tagung der Österreichischen Gesellschaft für Chirugie, May 20—23, 1970, Vienna, Verlag der Wiener Medizinischen Akademie, Vienna 1971.
58 L.B. Mellett and L.A. Woods, Cancer Res., 20 (1960) 524—532.
59 G.W. Aherne and M. Quinton, Cancer Treat. Rep., 65 (Suppl. 1) (1981) 55—60.
60 M.V. Freeman, J. Pharmacol. Exp. Ther., 120 (1957) 1—8.
61 S.G. Chakrabarti and I.A. Bernstein, Clin. Chem., 15 (1969) 1157—1161.
62 J.M. Kinkade, Jr., W.R. Vogler and P.G. Dayton, Biochem. Med., 10 (1974) 337—350.
63 S.P. Rothenberg, Anal. Biochem., 16 (1966) 176—179.
64 C. Bohuon, F. Duprey and C. Boudene, Clin. Chim. Acta, 57 (1974) 263—267.
65 L. Levine and E. Powers, Res. Commun. Chem. Pathol. Pharmacol., 9 (1974) 543—554.
66 V. Raso and R. Schreiber, Cancer Res., 35 (1975) 1407—1410.
67 L.J. Loeffler, M.R. Blum and M.A. Nelsen, Cancer Res., 36 (1976) 3306—3311.

68 J. Hendel, L.J. Sarek and E.F. Hvidberg, Clin. Chem., 22 (1976) 813—816.
69 G.W. Aherne, E.M. Piall and V. Marks, Brit. J. Cancer, 36 (1977) 608—617.
70 V. Raso, Cancer Treat. Rep., 61 (1977) 585—590.
71 J.W. Paxton and F.J. Rowell, Clin. Chim. Acta, 80 (1977) 563—572.
72 W. Aherne, E. Piall and V. Marks, Ann. Clin. Biochem., 15 (1978) 331—334.
73 J.W. Paxton, F.J. Rowell and G.M. Cree, Clin. Chem., 24 (1978) 1534—1538.
74 R.S. Kamel and J. Gardner, Clin. Chim. Acta, 89 (1978) 363—370.
75 R. Kamel, J. Landon and G.C. Forrest, Clin. Chem., 26 (1980) 97—100.
76 J.W. Paxton, Clin. Chem., 25 (1979) 491—492.
77 M.N. Al-Bassam, M.J. O'Sullivan, J.W. Bridges and V. Marks, Clin. Chem., 25 (1979) 1448—1452.
78 M. Oellerich, P. Engelhardt, M. Schaadt and V. Diehl, J. Clin. Chem. Clin. Biochem., 18 (1980) 169—174.
79 W.C. Werkheiser, S.F. Zakrzewski and C.A. Nichol, J. Pharmacol. Exp. Ther., 137 (1962) 162—166.
80 B. Overdijk, W.M.J. van der Kroef, A.A.M. Visser and G.J.M. Hooghwinkel, Clin. Chim. Acta, 59 (1975) 177—182.
81 E. Epstein, E.S. Baginski and B. Zac, Ann. Clin. Lab. Sci., 6 (1976) 312—317.
82 L.C. Falk, D.R. Clark, S.M. Kalman and T.F. Long, Clin. Chem., 22 (1976) 785—788.
83 P.R. Finley and R.J. Williams, Clin. Chem., 23 (1977) 2139—2141.
84 L.F. Brown, G.F. Johnson, D.L. Witte and R.D. Feld, Clin. Chem., 26 (1980) 335.
85 J.P. Persijn, J. Clin. Chem. Clin. Biochem., 17 (1979) 235—239.
86 J.L. Bock and R. Pierce, Clin. Chem., 26 (1980) 1510—1511.
87 C.E. Myers, M.E. Lippman, H.M. Eliot and B.A. Chabner, Proc. Nat. Acad. Sci. U.S., 72 (1975) 3683—3686.
88 E. Arons, S.P. Rothenberg, M. da Costa, C. Fischer and M.P. Iqbal, Cancer Res., 35 (1975) 2033—2038.
89 S.P. Rothenberg, M. da Costa and M.P. Iqbal, Cancer Treat. Rep., 61 (1977) 575—584.
90 R.J. Hayman, H. Fong and M.B. van der Weyden, J. Lab. Clin. Med., 93 (1979) 480—484.
91 W.P. Tong, J. Rosenberg and D.B. Ludlum, Lancet, ii (1975) 719.
92 D.C. Chatterji and J.F. Gallelli, J. Pharm. Sci., 66 (1977) 1219—1222.
93 J.L. Wisnicki, W.P. Tong and D.B. Ludlum, Cancer Treat. Rep., 62 (1978) 529—532.
94 Y.-M. Wang, S.K. Howell and J.A. Benvenuto, J. Liquid Chromatogr., 3 (1980) 1071—1078.
95 M.-L. Chen and W.L. Chiou, J. Chromatogr., 226 (1981) 125—134.
96 H. Breithaupt, E. Küenzlen and G. Goebel, Anal. Biochem., 121 (1982) 103—113.
97 Y.Y.Z. Farid, I.D. Watson and M.J. Stewart, J. Pharm. Biomed. Anal., 1 (1983) 55—63.
98 J.A. Nelson, B.A. Harris, W.J. Decker and D. Farquhar, Cancer Res., 37 (1977) 3970—3973.
99 J. Lankelma and H. Poppe, J. Chromatogr., 149 (1978) 587—598.
100 J. Lankelma and E. van der Klein and F. Ramaekers, Cancer Lett., 9 (1980) 133—142.
101 D.A. Cairnes and W.E. Evans, J. Chromatogr., 231 (1982) 103—110.
102 E. Watson, J.L. Cohen and K.K. Chan, Cancer Treat. Rep., 62 (1978) 381—387.
103 J.L. Cohen, G.H. Hisayasu, A.R. Barrientos, M.S.B. Nayar and K.K. Chan, J. Chromatogr., 181 (1980) 478—483.
104 G.J. Lawson, P.F. Dixon and G.W. Aherne, J. Chromatogr., 223 (1981) 225—231.
105 J. Dutrieu and Y.A. Delmotte, Fresenius' Z. Anal. Chem., 315 (1983) 539—542.
106 R.G. Buice and P. Sidhu, J. Pharm. Sci., 71 (1982) 74—76.
107 M. Przybylski, J. Preiss, R. Dennebaum and J. Fischer, Biomed. Mass Spectrom., 9 (1982) 22—32.
108 W.P. Tong, J.L. Wisnicki, J. Horton and D.B. Ludlum, Clin. Chim. Acta, 107 (1980) 67—72.

109 C. Canfell and W. Sadée, Cancer Treat. Rep., 64 (1980) 165—169.
110 O. Driessen, H. Beukers, L. Belfroid and A. Emonds, J. Chromatogr., 181 (1980) 441—448.
111 M. Anbar and G.A. St. John, Anal. Chem., 48 (1976) 198—203.
112 M.C. Kirk, W.C. Coburn, Jr., and J.R. Piper, Biomed. Mass Spectrom., 3 (1976) 245—247.
113 C.E. Hignite and D.L. Azarnoff, Biomed. Mass Spectrom., 5 (1978) 161—163.
114 Y.-M. Wang, E. Lantin and W.W. Sutow, Clin. Chem., 22 (1976) 1053—1056.
115 R.C. Donehower, K.R. Hande, J.C. Drake and B.A. Chabner, Clin. Pharmacol. Ther., 26 (1979) 63—72.
116 S.K. Howell, Y.-M. Wang, R. Hosoya and W.W. Sutow, Clin. Chem., 26 (1980) 734—737.
117 R.G. Buice, W.E. Evans, J. Karas, C.A. Nicholas, P. Sidhu, A.B. Straughn, M.C. Meyer and W.R. Crom, Clin. Chem., 26 (1980) 1902—1904.
118 S. Eksborg, Clin. Chem., 27 (1981) 1311—1312.
119 E.R. Garrett, G.H. Hurst and J.R. Green, Jr., J. Pharm. Sci., 66 (1977) 1422—1429.
120 A. Brandberg, O. Almersjö, A. Falsén, B. Gustavsson, L. Hafström and G.-B. Lindblom, Acta Pathol. Microbiol. Scand., 85 (1977) 227—234.
121 N. Kawabata, S. Sugiyama, T. Kuwamura, Y. Odaka and T. Satoh, J. Pharm. Sci., 72 (1983) 1162—1165.
122 A.P. De Leenheer, M.-C. Cosyns-Duyck and P.M. van Vaerenbergh, J. Pharm. Sci., 66 (1977) 1190—1193.
123 Y. Morimoto, M. Akimoto, K. Sugibayashi, T. Nadai and Y. Kato, Pharmazie, 36 (1981) 155—156.
124 O. Driessen, D. De Vos and P.J.A. Timmermans, J. Chromatogr., 162 (1979) 451—456.
125 E.A. de Bruijn, O. Driessen, N. van den Bosch, E. van Strijen, P.H.Th.J. Slee, A.T. van Oosterom and U.R. Tjaden, J. Chromatogr., 278 (1983) 283—289.
126 C. Pantarotto, A. Martini, G. Belvedere, A. Bossi, M.G. Donelli and A. Frigerio, J. Chromatogr., 99 (1974) 519—527.
127 K.V. Rao, K. Killion and Y. Tanrikut, J. Pharm. Sci., 63 (1974) 1328—1330.
128 B.L. Hillcoat, M. Kawai, P.B. McCulloch, J. Rosenfeld and C.K.O. Williams, Brit. J. Clin. Pharmacol., 3 (1976) 135—143.
129 R.E. Finch, M.R. Bending and A.F. Lant, J. Pharm. Sci., 67 (1978) 1489—1490.
130 H. Isomura, S. Higuchi and S. Kawamura, J. Chromatogr., 224 (1981) 423—429.
131 B.H. Min and W.A. Garland, Res. Commun. Chem. Pathol. Pharmacol., 22 (1978) 145—154.
132 J.P. Cano, J.P. Rigault, C. Aubert, Y. Carcassonne and J.F. Seitz, Bull. Cancer, 66 (1979) 67—73.
133 M.-C. Cosyns-Duyck, A.A.M. Cruyl, A.P. De Leenheer, A. De Schryver, J.V. Huys and F.M. Belpaire, Biomed. Mass Spectrom., 7 (1980) 61—64.
134 C. Aubert, J.P. Sommadossi, P. Coassolo, J.P. Cano and J.P. Rigault, Biomed. Mass Spectrom., 9 (1982) 336—339.
135 A.P. De Leenheer and M.C. Cosyns-Duyck, J. Pharm. Sci., 68 (1979) 1174—1176.
136 A.P. De Leenheer and M.C. Cosyns-Duyck, J. Chromatogr., 174 (1979) 325—339.
137 J.J. Windheuser, J.L. Sutter and E. Auen, J. Pharm. Sci., 61 (1972) 301—303.
138 J.L. Cohen and P.B. Brennan, J. Pharm. Sci., 62 (1973) 572—575.
139 C. Finn and W. Sadée, Cancer Chemother. Rep. Part 1, 59 (1975) 279—286.
140 A.T. Wu, H.-J. Schwandt, C. Finn and W. Sadée, Res. Commun. Chem. Pathol. Pharmacol., 14 (1976) 89—102.
141 D.B. Lakings, R.H. Adamson and R.B. Diasio, J. Chromatogr., 146 (1978) 512—517.
142 E.B. Hills, V.C. Godefroi, I.A. O'Leary, M. Burke, D. Andrzejewski, W. Brukwinski and J.P. Horwitz, J. Pharm. Sci., 66 (1977) 1497—1499.
143 T. Marunaka and Y. Umeno, J. Chromatogr., 221 (1980) 382—386.
144 H.W. van den Berg, R.F. Murphy, R. Hunter and D.T. Elmore, J. Chromatogr., 145 (1978) 311—314.

145 C. Pantarotto, R. Fanelli, S. Filippeschi, T. Facchinetti, F. Spreafico and M. Salmona, Anal. Biochem., 97 (1979) 232—238.
146 W.E. MacMillan, W.H. Wolberg and P.G. Welling, Cancer Res., 38 (1978) 3479—3482.
147 N. Christophidis, G. Mihaly, F. Vajda and W. Louis, Clin. Chem., 25 (1979) 83—86.
148 A.L. Buckpitt and M.R. Boyd, Anal. Biochem., 106 (1980) 432—437.
149 W.E. Wung and S.B. Howell, Clin. Chem., 26 (1980) 1704—1708.
150 C.L. Hornbeck, R.A. Floyd, J.C. Griffiths and J.E. Byfield, J. Pharm. Sci., 70 (1981) 1163—1166.
151 C.L. Hornbeck, J.C. Griffiths, R.A. Floyd, N.C. Ginther, J.E. Byfield and T.R. Sharp, Cancer Treat. Rep., 65 (1981) 69—72.
152 J.L.-S. Au, M.G. Wientjes, C.M. Luccioni and Y.M. Rustum, J. Chromatogr., 228 (1982) 245—256.
153 D.C. Sampson, R.M. Fox, M.H.N. Tattersall and W.J. Hensley, Ann. Clin. Biochem., 19 (1982) 125—128.
154 R.B. Diasio and M.E. Wilburn, J. Chromatogr. Sci., 17 (1979) 565—567.
155 C.F. Gelijkens and A.P. De Leenheer, J. Chromatogr., 194 (1980) 305—314.
156 A.A. Miller, J.A. Benvenuto and T.L. Loo, J. Chromatogr., 228 (1982) 165—176.
157 E.J. Quebbeman, N.E. Hoffman, A.A.R. Hamid and R.K. Ausman, J. Liq. Chromatogr., 7 (1984) 1489—1494.
158 D.S. Sitar, D.H. Shaw, Jr., M.P. Thirlwell and J.R. Ruedy, Cancer Res., 37 (1977) 3981—3984.
159 C. Aubert, C. Luccioni, P. Coassolo, J.P. Sommadossi and J.P. Cano, Arzneim.-Forsch., 31 (1981) 2048—2053.
160 J.L. Cohen and R.E. Brown, J. Chromatogr., 151 (1978) 237--276.
161 L.S.F. Hsu and T.C. Marrs, Ann. Clin. Biochem., 11 (1980) 272—276.
162 G.J. Peters, I. Kraal, E. Laurensse, A. Leyva and H.M. Pinedo, J. Chromatogr., 307 (1984) 464—468.
163 B. Gustavsson, A. Baldesten, P.-O. Hasselgren and O. Almersjö, J. Chromatogr., 179 (1979) 151—159.
164 B. Gustavsson, O. Almersjö, M. Berne and J. Waldenström, J. Chromatogr., 276 (1983) 395—401.
165 B.C. Baguley and E.-M. Falkenhaug, Cancer Chemother. Rep. Part 1, 55 (1971) 291—298.
166 B.C. Baguley and E.-M. Falkenhaug, Eur. J. Cancer, 11 (1975) 43—49.
167 R.L. Momparler, A. Labitan and M. Rossi, Cancer Res., 32 (1972) 408—412.
168 R.L. Furner, R.W. Gaston, J.D. Strobel, S. El Dareer and L.B. Mellett, J. Nat. Cancer Inst., 52 (1974) 1521—1528.
169 T. Okabayashi, S. Mihara and J.G. Moffatt, Cancer Res., 37 (1977) 625—628.
170 T. Okabayashi, S. Mihara, D.B. Repke and J.G. Moffatt, Cancer Res., 37 (1977) 619—624.
171 T. Okabayashi, S. Mihara, D.B. Repke and J.G. Moffatt, Cancer Res., 37 (1977) 3132—3135.
172 C. Pantarotto, A. Martini, G. Belvedere, A. Bossi, M.G. Donelli and A. Frigerio, J. Chromatogr., 99 (1974) 519—527.
173 J. Boutagy and D.J. Harvey, J. Chromatogr., 146 (1978) 283—296.
174 H. Breithaupt and J. Schick, J. Chromatogr., 225 (1981) 99—106.
175 A.L. Harris, C. Potter, C. Bunch, J. Boutagy, D.J. Harvey and D.G. Grahame-Smith, Brit. J. Clin. Pharmacol., 8 (1979) 219—227.
176 S.H. Wan, D.H. Huffman, D.L. Azarnoff, B. Hoogstraten and W.E. Larsen, Cancer Res., 34 (1974) 392—397.
177 W. Kreis, A. Greenspan, T. Woodcock and C. Gordon, J. Chromatogr. Sci., 14 (1976) 331—333.
178 W. Kreis, C. Gordon, C. Gizoni and T. Woodcock, Cancer Treat. Rep., 61 (1977) 643—646.
179 Y.M. Rustum, Anal. Biochem., 90 (1978) 289—299.
180 Y.M. Rustum and H.D. Preisler, Cancer Res., 39 (1979) 42—49.
181 M.G. Pallavicini and J.A. Mazrimas, J. Chromatogr., 183 (1980) 449—458.
182 R.W. Bury and P.J. Keary, J. Chromatogr., 146 (1978) 350—353.

183 P. Linssen, A. Drenthe-Schonk, H. Wessels and C. Haanen, J. Chromatogr., 223 (1981) 371—378.
184 G.G. Liversidge, T. Nishihata, T. Higuchi, R. Shaffer and M. Cortese, J. Chromatogr., 276 (1983) 375—383.
185 D.G. Bailey, T.W. Wilson and G.E. Johnson, J. Chromatogr., 111 (1975) 305—311.
186 T.L. Ding and L.Z. Benet, J. Chromatogr., 163 (1979) 281—288.
187 R.A. de Abreu, J.M. van Baal, T.J. Schouten and E.D.A.M. Schretlen and C.H.M.M. de Bruyn, J. Chromatogr., 227 (1982) 526—533.
188 R.E. Jonkers, B. Oosterhuis, R.J.M. ten Berge and C.J. van Boxtel, J. Chromatogr., 233 (1982) 249—255.
189 K. Tsutsumi, Y. Otsuki and T. Kinoshita, J. Chromatogr., 231 (1982) 393—399.
190 A.H. Chalmers, Biochem. Med., 12 (1975) 234—241.
191 J.M. Finkel, Anal. Biochem., 21 (1967) 362—371.
192 J.L. Maddocks, Brit. J. Clin. Pharmacol., 8 (1979) 273—278.
193 J.M. Finkel, J. Pharm. Sci., 64 (1975) 121—122.
194 A.D. Thomas, Talanta, 23 (1976) 393—386.
195 J.M. Rosenfeld, V.Y. Taguchi, B.L. Hillcoat and M. Kawai, Anal. Chem., 49 (1977) 725—727.
196 S. Floberg, P. Hartvig, B. Lindström, G. Lönnerholm and B. Odlind, J. Chromatogr., 225 (1981) 73—81.
197 A.F. Fell, S.M. Plag and J.M. Neil, J. Chromatogr., 186 (1979) 691—704.
198 T.P. Zimmerman, L.-C. Chu, C.J.L. Buggé, D.J. Nelson, G.M. Lyon and G.B. Elion, Cancer Res., 34 (1974) 221—224.
199 H.-J. Breter, B. Heicke, E.J. Zöllner and R.K. Zahn, Z. Klin. Chem. Klin. Biochem., 12 (1974) 223.
200 H.-J. Breter and R.K. Zahn, Z. Anal. Chem., 279 (1976) 151—152.
201 M.J. Harber and J.L. Maddocks, J. Chromatogr., 101 (1974) 231—234.
202 H.-J. Breter, Anal. Biochem., 80 (1977) 9—19.
203 H.-J. Breter, Z. Naturforsch. Teil C, 32 (1977) 905—907.
204 H.-J. Breter and R.K. Zahn, J. Chromatogr., 137 (1977) 61—68.
205 D.J. Nelson, C.J.L. Bugge, H.C. Krasny and T.P. Zimmerman, J. Chromatogr., 77 (1973) 181—190.
206 J.L. Day, L. Tterlikkis, R. Niemann, A. Mobley and C. Spikes, J. Pharm. Sci., 67 (1978) 1027—1028.
207 D.M. Tidd and S. Dedhar, J. Chromatogr., 145 (1978) 237—246.
208 P.K. Narang, R.L. Yeager and D.C. Chatterji, J. Chromatogr., 230 (1982) 373—380.
209 H. Imai, H. Yoshida, T. Masujima, I. Morita, K. Matsuura, A. Nakamaru, K. Katayama and H. Matsuo, Anal. Lett., 16 (B14) (1983) 1109—1120.
210 H. Breithaupt and G. Goebel, J. Chromatogr. Sci., 19 (1981) 496—499.
211 P.A. Andrews, M.J. Egorin, M.E. May and N.R. Bachur, J. Chromatogr., 227 (1982) 83—91.
212 N. Hobara and A. Watanabe, Acta Med. Okayama, 33 (1979) 239—243.
213 S.-N. Lin, K. Jessup, M. Floyd, T.-P.F. Wang, C.T. Van Buren, R.M. Caprioli and B.D. Kahan, Transplantation, 29 (1980) 290—294.
214 K. Lu, H.-Y. Yap and T.L. Loo, Cancer Res., 43 (1983) 1405—1408.
215 R.J. Owellen, C.A. Hartke and F.O. Hains, Cancer Res., 37 (1977) 2597—2602.
216 J.D. Teale, J.M. Clough and V. Marks, Brit. J. Clin. Pharmacol., 4 (1977) 169—172.
217 J.J. Langone, M.R. d'Onofrio and H. Van Vunakis, Anal. Biochem., 95 (1979) 214—221.
218 R.L. Nelson, R.W. Dyke and M.A. Root, Cancer Treat. Rev., 7 (Suppl.) (1980) 17—24.
219 V.S. Sethi, S.S. Burton and D.V. Jackson, Cancer Chemother. Pharmacol., 4 (1980) 183—187.
220 R.J. Owellen, M. Blair, A. Van Tosh and F.C. Hains, Cancer Treat. Rep., 65 (1981) 469—475.
221 J.B.A. Kipp and G.W. Barendsen, Acta Pathol. Microbiol. Scand. Sect. A, 274 (Suppl.) (1981) 531—532.
222 J.B.A. Kipp and G.W. Barendsen, Eur. J. Cancer Clin. Oncol., 17 (1981) 867—873.

223 M.P. Hacker, J.R. Dank and W.B. Ershler, Cancer Res., 44 (1984) 478—481.
224 W.P. van Bennekom, U.R. Tjaden, E.A. de Bruijn and A.T. van Oosterom, Anal. Chim. Acta, 156 (1984) 289—294.
225 K. Fujiwara, H. Saikusa, M. Yasuno and T. Kitagawa, Cancer Res., 42 (1982) 1487—1491.
226 U.R. Tjaden, J.P. Langenberg, K. Ensing and W.P. van Bennekom, E.A. de Bruijn and A.T. van Oosterom, J. Chromatogr., 232 (1982) 355—367.
227 D. Edwards, A.B. Selkirk and R.B. Taylor, Int. J. Pharm., 4 (1979) 21—26.
228 S. Eksborg, H. Ehrsson and A. Lindfors, J. Chromatogr., 274 (1983) 263—270.
229 A. Kono, Y. Hara, S. Eguchi, M. Tanaka and Y. Matsushima, J. Chromatogr., 164 (1979) 404—406.
230 J. den Hartigh, W.J. van Oort, M.C.Y.M. Bocken and H.M. Pinedo, Anal. Chim. Acta, 127 (1981) 47—53.
231 J.W. Gyves, W.D. Ensminger, D. VanHarken, J. Niederhuber, P. Stetson and S. Walker, Clin. Pharmacol. Ther., 34 (1983) 259—265.
232 G.A. van Hazel and J.S. Kovach, Cancer Chemother. Pharmacol., 8 (1982) 189—192.
233 P.A. Andrews, S.-S. Pan and N.R. Bachur, J. Chromatogr., 262 (1983) 231—247.
234 W. Greene, D. Huffman, P.H. Wiernik, S. Schimpff, R. Benjamin and N. Bachur, Cancer, 30 (1972) 1419—1427.
235 S. Eksborg, H. Ehrsson, I. Wallin and A. Lindfors, Acta Pharm. Suec., 18 (1981) 215—220.
236 S. Eksborg and H. Ehrsson, J. Pharm. Biomed. Anal., 2 (1985) 297—303.
237 M.J.M. Oosterbaan, R.J.M. Dirks, T.B. Vree and E. van der Kleijn, J. Chromatogr., 306 (1984) 323—332.
238 J.M. Finkel, K.T. Knapp and L.T. Mulligan, Cancer Chemother.Rep. Part 1, 53 (1969) 159—164.
239 R. Rosso, C. Ravazzoni, M. Esposito, R. Sala and L. Santi, Eur. J. Cancer, 8 (1972) 455—459.
240 N.R. Bachur, A.L. Moore, J.G. Bernstein and A. Liu, Cancer Chem. Rep. Part 1, 54 (1970) 89—94.
241 H.S. Schwartz, Biochem. Med., 7 (1973) 396—404.
242 M.J. Egorin, R.C. Hildebrand, E.F. Cimino and N.R. Bachur, Cancer Res., 34 (1974) 2243—2245.
243 A. Krishan, M. Israel, E.J. Modest and E. Frei, III, Cancer Res., 36 (1976) 2114—2116.
244 L. Bigotte, B. Arvidson and Y. Olsson, Acta Neuropathol., 57 (1982) 121—129.
245 G. Nicolð, M. Esposito, A. Belcastro, S. Rovida, E. Margallo and L. Santi, Anal. Quant. Cytol., 4 (1982) 295—301.
246 A. Krishan and R. Ganapathi, J. Histochem. Cytochem., 27 (1979) 1655—1656.
247 P. Sonneveld and G.J. van den Engh, Leukemia Res., 5 (1981) 251—257.
248 A. Garner and B.W. Fox, Chem.-Biol. Interact., 36 (1981) 189—210.
249 H. Tapiero, A. Fourcade, P. Vaigot and J.J. Farhi, Cytometry, 2 (1982) 298—302.
250 G.M. Rao, J.W. Lown and J.A. Plambeck, J. Electrochem. Soc., 125 (1978) 534—539.
251 C. Molinier-Jumel, B. Malfoy, J.A. Reynaud and G. Aubel-Sadron, Biochem. Biophys. Res. Commun., 84 (1978) 441—449.
252 L.A. Sternson and G. Thomas, Anal. Lett., 10 (1977) 99—109.
253 R.P. Baldwin, D. Packett and T.M. Woodcock, Anal. Chem., 53 (1981) 540—542.
254 E.N. Chaney, Jr. and R.P. Baldwin, Anal. Chem., 54 (1982) 2556—2560.
255 H. Van Vunakis, J.J. Langone, L.J. Riceberg and L. Levine, Cancer Res., 34 (1974) 2546—2552.
256 E. Piall, G.W. Aherne and V. Marks, Clin. Chem., 28 (1982) 119—121.
257 R. Rahmani, P. Gil, M. Martin, A. Durand, J. Barbet and J.-P. Cano, J. Pharm. Biomed. Anal., 1 (1983) 301—309.
258 N.R. Bachur, C.E. Riggs, Jr., M.R. Green, J.J. Langone, H. Van Vunakis and L. Levine, Clin. Pharm. Ther., 21 (1976) 70—77.
259 K. Fujiwara, M. Yasuno and T. Kitagawa, J. Immunol. Methods, 45 (1981) 195—203.
260 K. Hirano, T. Nagae, T. Adachi, Y. Ito and M. Sugiura, J. Pharm. Dyn., 6 (1983) 588—594.

261 N.R. Bachur, J. Pharmacol. Exp. Ther., 177 (1971) 573—578.
262 N.R. Bachur, R.C. Hildebrand and R.S. Jaenke, J. Pharmacol. Exp. Ther., 191 (1974) 331—340.
263 R.S. Benjamin, C.E. Riggs, Jr. and N.R. Bachur, Cancer Res., 37 (1977) 1416—1420.
264 K.K. Chan and P.A. Harris, Res. Commun. Chem. Pathol. Pharmacol., 6 (1973) 447—463.
265 E. Watson and K.K. Chan, Cancer Treat. Rep., 60 (1976) 1611—1618.
266 T. Gessner, J. Robert, W. Bolanowska, B. Hoerni, M. Durand, H. Preisler and J. Rustum, J. Med., 12 (1981) 183—193.
267 J.H. Peters and J.F. Murray, Jr., J. Liquid Chromatogr., 2 (1979) 45—52.
268 S.D. Averbuch, T.T. Finkelstein, S.E. Fandrich and S.D. Reich, J. Pharm. Sci., 70 (1981) 265—269.
269 A. Zenebergh, R. Baurain and A. Trouet, Cancer Chemother. Pharmacol., 8 (1982) 243—249.
270 R. Erttmann, J. Chromatogr., 277 (1983) 433—435.
271 S.E. Fandrich and K.A. Pittman, J. Chromatogr., 223 (1981) 155—164.
272 J. Lankelma, P.G.M. Penders, J.G. McVie, A. Leyva, W.W. ten Bokkel Huinink, M.M. de Planque and H.M. Pinedo, Eur. J. Cancer Clin. Oncol., 18 (1982) 363—367.
273 R. Baurain, D. Deprez-De Campeneere and A. Trouet, Cancer Chemother. Pharmacol., 2 (1979) 37—41.
274 R. Hulhoven, J.P. Desager and C. Harvengt, Cancer Chemother. Pharmacol., 3 (1979) 133.
275 B. Leyland-Jones, P. Deesen, R. Wittes and C.W. Young, Clin. Pharmacol. Ther., 33 (1982) 244.
276 E. Moro, M.G. Jannuzzo, M. Ranghieri, S. Stegnjaich and G. Valzelli, J. Chromatogr., 230 (1982) 207—211.
277 P.E. Deesen and B. Leyland-Jones, Drug Metab. Dispos., 12 (1984) 9—13.
278 M. Israel, W.J. Pegg and P.M. Wilkinson, J. Pharmacol. Exp. Ther., 204 (1978) 696—701.
279 E. Moro, V. Bellotti, M.G. Jannuzzo, S. Stegnjaich and G. Valzelli, J. Chromatogr., 274 (1983) 281—287.
280 R. Hulhoven and J.P. Desager, J. Chromatogr., 125 (1976) 369—374.
281 M. Israel, W.J. Pegg, P.M. Wilkinson and M.B. Garnick, J. Liquid Chromatogr., 1 (1978) 795—809.
282 C. Paul, R. Baurain, G. Gahrton and C. Peterson, Cancer Lett., 9 (1980) 263—269.
283 J.J. Langone, H. Van Vunakis and N.R. Bachur, Biochem. Med., 12 (1975) 283—289.
284 S. Eksborg, H. Ehrsson, B. Andersson and M. Beran, J. Chromatogr., 153 (1978) 211—218.
285 R. Baurain, D. Deprez-De Campeneere and A. Trouet, Anal. Biochem., 94 (1979) 112—116.
286 S. Eksborg, H. Ehrsson and I. Andersson, J. Chromatogr., 164 (1979) 479—486.
287 R.N. Pierce and P.I. Jatlow, J. Chromatogr., 164 (1979) 471—478.
288 P.A. Andrews, D.E. Brenner, F.-T.E. Chou, H. Kubo and N.R. Bachur, Drug Metab. Dispos., 8 (1980) 152—156.
289 A.M.B. Bots, W.J. van Oort, J. Noordhoek, A. van Dijk, S.W. Klein and Q.G.C.M. van Hoesel, J. Chromatogr., 272 (1983) 421—427.
290 J.F. Strauss, R.L. Kitchens, V.W. Patrizi and E.P. Frenkel, J. Chromatogr., 221 (1980) 139—144.
291 S. Shinozawa, Y. Mimaki, Y. Araki and T. Oda, J. Chromatogr., 196 (1980) 463—469.
292 C. Akpofure, C.A. Riley, J.A. Sinkule and W.E. Evans, J. Chromatogr., 232 (1982) 377—383.
293 A.N. Kotake, N.J. Vogelzang, R.A. Larson and N. Choporis, J. Chromatogr., 337 (1985) 194—200.
294 J.E. Brown, P.A. Wilkinson and J.R. Brown, J. Chromatogr., 226 (1981) 521—525.
295 W. Bolanowska, T. Gessner and H. Preisler, Cancer Chemother. Pharmacol., 10 (1983) 187—191.
296 M.J. Sepaniak and E.S. Yeung, J. Chromatogr., 190 (1980) 377—383.

297 S. Eksborg, J. Pharm. Sci., 67 (1978) 782—785.
298 J. Robert, J. Liquid Chromatogr., 3 (1980) 1561—1572.
299 H.G. Barth and A.Z. Conner, J. Chromatogr., 131 (1977) 375—381.
300 A.C. Haneke, J. Crawford and A. Aszalos, J. Pharm. Sci., 70 (1981) 1112—1115.
301 S. Eksborg, J. Chromatogr., 149 (1978) 225—232.
302 M.A. Van Lancker, H.J.C.F. Nelis and A.P. De Leenheer, J. Chromatogr., 254 (1983) 45—52.
303 H. Brockmann Jr., H. Budzikiewicz, C. Djerassi, H. Brockmann and J. Niemeyer, Chem. Ber., 98 (1965) 1260—1269.
304 A. Vigevani, B. Gioia and G. Cassinelli, Carbohydr. Res., 32 (1974) 321—330.
305 P.P. Roller, M. Sutphin and A.A. Aszalos, Biomed. Mass Spectrom., 3 (1976) 166—171.
306 E. Watson and K.K. Chan, J. Pharm. Sci., 67 (1978) 1243—1246.
307 K.K. Chan and E. Watson, J. Pharm. Sci., 67 (1978) 1748—1752.
308 K.H. Maurer and U. Rapp, Advan. Mass Spectr. Biochem. Med., 1 (1976) 541—551.
309 B. Gioia, E. Arlandini and A. Vigevani, Biomed. Mass Spectrom., 11 (1984) 35—40.
310 H. Nakazawa, P.A. Andrews, N.R. Bachur and Y. Ito, J. Chromatogr., 205 (1981) 482—485.
311 H. Akedo and K. Shinkai, J. Chromatogr., 227 (1982) 262—265.
312 Y.-M. Peng, D. Ormberg, D.S. Alberts and T.P. Davis, J. Chromatogr., 233 (1982) 235—247.
313 D.L. Reynolds, L.A. Sternson and A.J. Repta, J. Chromatogr., 222 (1981) 225—240.
314 G. Powis, J. Chromatogr., 226 (1981) 514—520.
315 R.F. Taylor and L.A. Gaudio, J. Chromatogr., 187 (1980) 212—217.
316 F. Ostroy and R.A. Gams, J. Liquid Chromatogr., 3 (1980) 637—644.
317 S.R. Cox, Diss. Abstr. Int., 41 (1981) 3746.
318 Y.-M. Peng, T.P. Davis and D.S. Alberts, Life Sci., 29 (1981) 361—369.
319 S.T. Houpt and R.P. Baldwin, Anal. Lett., 16 (1983) 1343—1354.
320 K. Lu, N. Savaraj, M.T. Huang, D. Moore and T.L. Loo, J. Liquid Chromatogr., 5 (1982) 1323—1328.
321 P.E. Gormley and R.L. Cysyk, Anal. Biochem., 96 (1979) 504—507.
322 A. Emonds, O. Driessen, E.A. de Bruijn and A.T. van Oosterom, Fresenius' Z. Anal. Chem., 307 (1981) 286—287.
323 L. Malspeis, M.N. Khan and H.B. Blat, Proc. Amer. Ass. Cancer Res., 20 (1979) 384.
324 J.L. Jurlina and J.W. Paxton, J. Chromatogr., 276 (1983) 367—374.
325 W.J. Rzeszotarski, W.C. Eckelman and R.C. Reba, J. Chromatogr., 124 (1976) 88—91.
326 C.R. Williams, Proc. Anal. Div. Chem. Soc., 14 (1977) 242—244.
327 T.T. Sakai, J. Chromatogr., 161 (1978) 389—392.
328 M. Brightwell, J. Chromatogr., 170 (1979) 443—445.
329 T.T. Sakai and J.M. Riordan, J. Chromatogr., 178 (1979) 302—306.
330 A.H. Thomas, P. Newland and N.R. Sharma, J. Chromatogr., 291 (1984) 219—230.
331 N. Thakrar and K.T. Douglas, Cancer Lett., 13 (1981) 265—268.
332 A. Broughton and J.E. Strong, Cancer Res., 36 (1976) 1418—1421.
333 M.K. Elson, M.M. Oken and R.B. Shafer, J. Nucl. Med., 18 (1977) 296—299.
334 J.E. Strong, A. Broughton and S.T. Crooke, Cancer Treat. Rep., 61 (1977) 1509—1512.
335 G.W. Aherne, N. Saesow, S. James and V. Marks, Cancer Treat. Rep., 66 (1982) 1365—1370.
336 K. Fujiwara, M. Yasuno and T. Kitagawa, Cancer Res., 41 (1981) 4121—4126.
337 K. Fujiwara, M. Isobe, H. Saikusa, H. Nakamura, T. Kitagawa and S. Takahashi, Cancer Treat. Rep., 67 (1983) 363—369.
338 G.K. Shiu, T.J. Goehl and W.H. Pitlick, J. Pharm. Sci., 68 (1979) 232—234.
339 G.K. Shiu and T.J. Goehl, J. Chromatogr., 181 (1980) 127—131.
340 D.F. Long, A.J. Repta and L.A. Sternson, Int. J. Pharm., 6 (1980) 167—173.
341 C.M. Riley, L.A. Sternson, A.J. Repta and R.W. Siegler, J. Chromatogr., 229 (1982) 373—386.
342 R.G. Miller and J.U. Doerger, At. Absorpt. Newsl., 14 (1975) 66—67.
343 A.H. Jones, Anal. Chem., 48 (1976) 1472—1474.
344 C.L. Litterst, T.E. Gram, R.L. Dedrick, A.F. Leroy and A.M. Guarino, Cancer Res., 36 (1976) 2340—2344.

345 M.F. Pera, Jr. and H.C. Harder, Clin. Chem., 23 (1977) 1245—1249.
346 A.F. LeRoy, M.L. Wehling, H.L. Sponseller, W.S. Friauf, R.E. Solomon, R.L. Dedrick, C.L. Litterst, T.E. Gram, A.M. Guarino and D.A. Becker, Biochem. Med. 18 (1977) 184—191.
347 P.E. Gormley, J.M. Bull, A.F. LeRoy and R. Cysyk, Clin. Pharmacol. Ther., 25 (1979) 351—357.
348 J.B. Vermorken, W.J.F. van der Vijgh and H.M. Pinedo, Res. Commun. Chem. Pathol. Pharmacol., 28 (1980) 319—328.
349 J. Smeyers-Verbeke, M.R. Detaevernier, L. Denis and D.L. Massart, Clin. Chim. Acta, 113 (1981) 329—333.
350 D. Priesner, L.A. Sternson and A.J. Repta, Anal. Lett., 14 (1981) 1255—1268.
351 S.J. Bannister, L.A. Sternson, A.J. Repta and G.W. James, Clin. Chem., 23 (1977) 2258—2262.
352 S.J. Bannister, Y. Chang, L.A. Sternson and A.J. Repta, Clin. Chem., 24 (1978) 877—880.
353 T.F. Patton, K.J. Himmelstein, R. Belt, S.J. Bannister, L.A. Sternson and A.J. Repta, Cancer Treat. Rep., 62 (1978) 1359—1362.
354 D.A. Hull, N. Muhammad, J.G. Lanese, S.D. Reich, T.T. Finkelstein and S. Fandrich, J. Pharm. Sci., 70 (1981) 500—502.
355 M.L. Denniston, L.A. Sternson and A.J. Repta, Anal. Lett., 14 (1981) 451—462.
356 I. Bartosek and M.T. Cattaneo, in P. Periti and G.G. Grassi (Editors), Curr. Chem. Immunother., Proc. 12th International Congress of Chemotherapy, Vol. II, Florence, July 19—21, 1981, The American Society for Microbiology, Washington, DC, 1981, pp. 1382—1383.
357 I. Bartosek, M.T. Cattaneo, G. Grasselli, A. Guaitani, R. Urso, E. Zucca, A. Libretti and S. Garattini, Tumori, 69 (1983) 395—402.
358 C.M. Riley, L.A. Sternson and A.J. Repta, J. Chromatogr., 217 (1981) 405—420.
359 C.M. Riley, L.A. Sternson and A.J. Repta, J. Chromatogr., 219 (1981) 235—244.
360 C.M. Riley, L.A. Sternson and A.J. Repta, J. Pharm. Sci., 72 (1983) 351—355.
361 S.J. Bannister, L.A. Sternson and A.J. Repta, J. Chromatogr., 273 (1983) 301—318.
362 I.S. Krull, X.-D. Ding, S. Braverman, C. Selavka, F. Hochberg and L.A. Sternson, J. Chromatogr. Sci., 21 (1983) 166—173.
363 Y. Chang, L.A. Sternson and A.J. Repta, Anal. Lett., 11 (1978) 449—459.
364 K.J. Himmelstein, T.F. Patton, R.J. Belt, S. Taylor, A.J. Repta and L.A. Sternson, Clin. Pharmacol. Ther., 29 (1981) 658—664.
365 S.J. Bannister, L.A. Sternson and A.J. Repta, J. Chromatogr., 173 (1979) 333—342.
366 O.H. Drummer, A. Proudfoot, L. Howes and W.J. Louis, Clin. Chim. Acta, 136 (1984) 65—74.
367 P.A. Reece, J.T. McCall, G. Powis and R.L. Richardson, J. Chromatogr., 306 (1984) 417—423.
368 R.F. Borch, J.H. Markovitz and M.E. Pleasants, Anal. Lett., 12 (1979) 917—926.
369 K.C. Marsh, L.A. Sternson and A.J. Repta, Anal. Chem., 56 (1984) 491—497.
370 A.A. Hincal, D.F. Long and A.J. Repta, J. Parent. Drug Ass., 33 (1979) 107—116.
371 R.J. Strife, I. Jardine and M. Colvin, J. Chromatogr., 182 (1980) 211—220.
372 L.M. Allen, J. Pharm. Sci., 69 (1980) 1440—1441.
373 P. Farina, G. Marzillo and M. d'Incalci, J. Chromatogr., 222 (1981) 141—145.
374 R.J. Strife, I. Jardine and M. Colvin, J. Chromatogr., 224 (1981) 168—174.
375 J.J.M. Holthuis, W.J. van Oort and H.M. Pinedo, Anal. Chim. Acta, 130 (1981) 23—30.
376 C.E. Werkhoven-Goewie, U.A.T. Brinkman, R.W. Frei, C. de Ruiter and J. de Vries, J. Chromatogr., 276 (1983) 349—357.
377 J.J.M. Holthuis, F.M.G.M. Römkens, H.M. Pinedo and W.J. van Oort, J. Pharm. Biomed. Anal., 1 (1983) 89—97.
378 V.A. Levin, W. Hoffman and R.J. Weinkam, Cancer Treat. Rep., 62 (1978) 1305—1312.
379 D.C. Chatterji, R.F. Greene and J.F. Gallelli, J. Pharm. Sci., 67 (1978) 1527—1532.
380 R.G. Smith, S.C. Blackstock, L.K. Cheung and T.L. Loo, Anal. Chem., 53 (1981) 1205—1208.
381 I.S. Krull, J. Strauss, F. Hochberg and N.T. Zervas, J. Anal. Toxicol., 5 (1981) 42—46.
382 R.L. Yeager, E.H. Oldfield and D.C. Chatterji, J. Chromatogr., 305 (1984) 496—501.
383 B. Caddy, O.R. Idowu and J.F. Stuart, Ther. Drug Monitor., 4 (1982) 389—395.
384 R.J. Weinkam, J.H.C. Wen, D.E. Furst and V.A. Levin, Clin. Chem., 24 (1978) 45—49.