Journal of Food and Drug Analysis
1996. 4(4) : 271-292

Stability-Indicating High-Performance Liquid Chromatographic Assay Methods for Drugs in Pharmaceutical Dosage Forms: Part I

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ABSTRACT

A review of applications of stability-indicating high-performance liquid chromatographic (HPLC) assay methods for drugs in various pharmaceutical dosage forms is presented. A survey of articles primarily on the significant developments in this field is covered in this paper. Selected substances are grouped to integrate drugs that share similar uses or actions. Papers selected by the authors are briefly summarized, and HPLC systems developed in these papers for stability testing of pharmaceuticals are tabulated to assist readers in selecting an appropriate system for a given pharmaceutical. The information given in the table includes the drug names, materials, column types, mobile-phase compositions, detections, internal standards used and the literature references.

Key words: Pharmaceutical dosage forms, stability-indicating, high-performance liquid chromatography(HPLC).

INTRODUCTION

The term "stability" of a drug dosage form refers to the ability of a particular formulation, in a specific container, to maintain its physical, chemical, therapeutic, and toxicological specifications presented in the monograph on identity, strength, quality, and purity. The stability of a drug product should ordinarily be demonstrated by its manufacturer by methods appropriate for the purpose[1-4]. Obviously, a stability testing problem is never simple[5].

It is well known that pharmaceutical analysts are always confronted with the tasks of developing analytical methods for stability studies of drugs. Nevertheless, nonspecific assay techniques can often result in unreliable measurements of drug stability[6]. A stability-indicating method which can selectively separate each active ingredient from its degradation products, process impurities, and formulation excipients, so that the intact drug molecule can be accurately determined, is paramount for stability testing.

HPLC was developed during the 1960s and improved during the 1970s. The use of HPLC in the determination of pharmaceuticals either as a

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Accepted for Publication: Sep. 20, 1996

final step of measurement or as a separation technique has undergone an enormous expansion during the 1980s, through technological innovation. Currently, HPLC is widely used in the stability testings of pharmaceuticals.

Six special requirements for an HPLC method which may be used for stability testings and therefore be defined as a stability-indicating method can be summarized as follows[7]:

(1) The peak of the main component (drug substance) should not co-elute with any other peaks originating from its production (by-products) or formed by decomposition (degradation products); the method should be able to detect the decrease in the active content throughout the period of the stability investigations (stability-indicating assay method).

(2) Desired resolutions between the peak of the main component and adjacent peak pairs can be selected to be higher than in other instances to identify possible degradation products similar in structure and chromatographic characteristics, and formed during various storage conditions at low concentrations (stability-indicating purity testing method).

(3) The optimum k' value for the main component is between 5 and 8 to achieve the necessary band spacing for the possible decomposition products with different chemical natures.

(4) The desired value of the precision of stability-indicating assay methods should not exceed +1.0%, in order that small decreases in active content can be accurately measured.

(5) The peak(s) of decomposition product(s) with different chemical natures should be separated from those of impurities present in the sample at the outset of the investigations, as the results of assay and purity tests are evaluated together and can be corrected using the original impurity content.

(6) Peaks of secondary decomposition products (formed by degradation of by-products and/or decomposition products) can also be separated from other peaks.

Hence, a stability-indicating HPLC method should be specific at the time the drug substance is determined by itself, the drug substance content of a dosage form is assayed, and the dosage form has aged. Also, it should be sufficiently sensitive to detect all low levels of components. Furthermore, a stability-indicating HPLC method is important in the study of drug decompositions which can be the criterion for product acceptance, and must be verified and not assumed.

Thousands of papers have been published on the stability of pharmaceuticals, however, the present review focuses exclusively on articles primarily related to the stability-indicating HPLC assay methods of pharmaceuticals. The substances discussed in this review are grouped to assemble those drugs that have similar uses or actions.

In addition, the stability-indicating HPLC systems which are conventionally employed for stability testing of pharmaceuticals are tabulated as an aid in selecting an appropriate system for a given pharmaceutical. The information given in Table 1 includes the common names of drugs, column types, mobile-phase compositions, the wavelength used and the literature reference.

This is not a comprehensive review of all published papers, since we have selected only those papers which, in our opinion, appear more relevant to this topic. A total of 189 references were selected for brief comment here. We have divided this review article into two parts. Part I includes Abstract, Introduction, Published Reviews and some of the Brief Summaries. Part II, which will appear in the March (1997) issue of this journal, continues to summarize the remaining references selected by the authors. Any comments or suggestions concerning this review are welcomed.

PUBLISHED REVIEWS

Since 1970, there have been several noteworthy reviews related to the topics of stability-indicating methods. Chafetz[8] extensively reviewed the importance of analytical methodology in drug decomposition. A critical examination of assay methods for representative drugs with respect to

their usefulness in establishing and monitoring drug stability was provided, and a critical review of some methods for determination of stability was presented.

Mollica et al.[9] reviewed the many facets of stability and in addition, included rates, mechanisms and pathways of degradation; dosage forms; marketed product stability; and regulatory considerations.

The review by Taylor and Shivji[10] discussed commonly adopted criteria for a stability-indicating assay and the merits of choosing a reactant or decomposition product for monitoring decomposition. Examples of the application of the initial-rate method to simple and complex drug decomposition systems for the determination of decomposition rate constants were also provided in this review.

Metha[11] attempted to identify the issues that are critical in the development of an analytical method for the stability testing of drugs in solution. He presented a short review of the analytical techniques used in stability test and emphasized the requirements for a stability-indicating analytical method to carry out such tests.

Boehlert[12] discussed a variety of analytical methods used in drug stability studies and presented the need for validation of these methods, emphasizing evaluation of specificity, linearity, precision, accuracy, sensitivity and ruggedness. Illustration of the development of stability-indicating assays is provided, utilizing 5 different drugs representing various types of problems.

Kumar and Sunder[13] described various stability-indicating tests, including titrimetry, spectrophotometry, colorimetry and chromatography.

Kingsford[14] presented recommendations in designing drug stability trials, in an effort to improve methods currently in use. These include choice of a suitable stability-indicating assay, assay precision, temperature factors relevant to the application of the Arrhenius equation to yield precise results, application of the Arrhenius equation to the drug and the question of whether the accelerated stability trial examines the same degradation reaction observed at room temperature .

Carstensen and Rhodes[15] discussed rational policies representative of academia, the pharmaceutical industry, the U. S. Food and Drug Administration, and the U. S. Pharmacopoeia for stability testing, including what parameters should be monitored in the stability testing of pharmaceutical products, stability-indicating assays, retained sample stability testing, fixed date method of stability testing, and stability testing by evaluation of market samples.

Dong et al.[16] presented a review, which emphasizes the method development and drug evaluation and with reference to U. S. P. XXII 1990 method validation guidelines and the use of modern LC software instrumentation and diode-array detection.

BRIEF SUMMARIES

I. *Analgesic and Anti-inflammatory Agents*

Hsu et al. of National Laboratories of Foods and Drugs (NLFD) in Taiwan[17] have developed a rapid, sensitive and precise method for the analysis of large numbers of samples containing ketoprofen. Acid, base and photodegradations of the drug were also carried out in this study and different chromatograms were obtained.

Carprofen in solid dosage forms and in bulk material, and dichlorophen and a trimer impurity in raw material and veterinary capsules were successfully determined by Ross et al.[18] and Shah et al.[19], respectively.

Burce and Boehlert[20] developed a stability-indicating assay for the degradation products of procarbazine HCl using HPLC and reported that the typical range for degradation products in capsules is 0.1-0.5% up to 4.5 years.

The determination of chlorpromazine and its degradation products in pharmaceutical dosage forms was presented by Chagonda and Millership [21].

Kubala et al.[22] determined diclofenac sodium in raw materials and pharmaceutical solid dosage forms. Diclofenac sodium and six related

compounds (potential impurities and degradation products) can be separated in approximately 43 min. 1-(2,6-dichlorophenyl)-2-indolin-2-one was detected as a degradation product under accelerated aging conditions (20 days at 90°C and 55% r. h.).

HPLC procedures were developed for the determination of some anti-inflammatory drugs incorporated in gel ointments, indomethacin in the controlled-release suppositories, sulindac in tablet formulations, and nefopam hydrochloride and its degradation products by Yamamura et al.[23], Ping et al.[24], Jalal et al.[25] and Tu et al.[27], respectively. All these papers studied the stability of the cited drugs and reported that common excipients and/or degradation products yielded no interference.

Using HPLC, Kaffenberger et al.[26] determined tebufelone [3,5-di-t-butyl-4-hydroxyphenyl pent-4-ynyl ketone] (a new anti-inflammatory drug) strength and studied its stability in bulk drug, dosage formulations and feed admixtures. Recoveries of tebufelone from bulk drug and capsules, and from feed mixtures were >99% and 96 to 102%, respectively.

Fabre et al.[28] simultaneously determined Oxyphenbutazone and six potential decomposition products by HPLC. The method was more sensitive than TLC and allowed the determination of 0.1% of each degradation product. The technique was applied to the analysis of ointments, tablets and capsules.

II. *Anti-arrhythmic Agents*

Das-Gupta[29] developed an HPLC procedure for the quantitation of verapamil hydrochloride. The stability of the drug was studied at a range of pH values, and samples decomposed by heating in 0.33M-NaOH for approximately 50 min. were also analyzed.

III. *Antibiotics*

Hsu and Cheng[30] of NLFD successively quantified cloxacillin in commercial preparations and for stability studies. Thermal degradation products did not interfere with the determination. Latter, Hsu and Fann[31] determined dicloxacillin preparations by another HPLC method which is suitable for potency assays and stability studies. Their results compared well with those of the official microbiological method.

Pavli and Sokolic[32] comparatively determined bacitracin by HPLC and microbiological methods described in B. P. 1980 in some pharmaceuticals and [animal] feed grade preparations. HPLC yielded more useful analytical information, and was particularly useful for rapid and precise determination of bacitracin A, B1 and B2. The microbiological method was found to be less selective. HPLC indicates that bacitracin B1 is composed of two components, and can be used to determine bacitracin F in stability studies.

In connection with the stability and compatibility studies of cephamandole nafate with PVC infusion bags, Faouzi et al.[33] applied an HPLC method developed for the determination of the drug and its hydrolysis product cephamandole in infusion solution of 5% glucose or 0.9% NaCl. No significant drug loss was found during simulated infusions for 1 h using PVC infusion bags and administration sets, and no significant differences were found between the two infusion solutions. The stability of the drug (without protection from light) when stored at room temperature for 24 h and at 4°C for 7 days is discussed.

The stability of metronidazole benzoate [benzoyl metronidazole] in liquid preparations and norfloxacin in aqueous solution were investigated by Sa'-sa et al.[34] and Nangia et al.[35], respectively, using HPLC. The former drug was found to be most stable at pH 4.

A stability-indicating HPLC method for norfloxacin in tablets using PSDVB [poly(styrene-divinylbenzene)-]based stationary phase was developed by Rotar and Lampic[36]. The intra- and inter-day coefficients of variation (n = 5) were 1.6 and 2%, respectively. On the other hand, Chen et al.[37] described an HPLC method for the determination of norfloxacin glutamate and glucuronate in solid and liquid dosage forms. The

method was used in thermal stability studies; the shelf-life of the solid and liquid products was 2 and 1.5 years, respectively.

Pan[38] developed a reversed-phase HPLC method for the determination cefoperazone sodium in several common fluids and applied in stability testing, and De-Schutter et al.[40] described a reversed-phase ion-pair high-performance liquid chromatographic analysis of tetryzoline [tetrahydrozoline] hydrochloride in nasal preparations.

Wang and Yeh of National Defense Medical Center (NDMC) in Taiwan[39] used an stability-indicating HPLC method to assay cephazolin in pharmaceutical dosage forms.

An HPLC stability-indicating assay for cefuroxime sodium solution in relation to clinical practice was studied by Jackson and Perrett[41]. The method was used to analyze freshly prepared and degraded samples, with degradation being indicated by a reduction in peak height. The degradation reaction was first order and independent of pH.

An HPLC procedure for the measurement of ceftazidime arginine in aqueous solution was developed by Nahata and Morosco[42]. The method was applied to evaluation of storage stability of ceftazidime in plastic syringes.

Klimes and Zahradnicek[43] selected chromatographic conditions to isolate and identify hydrolytic decomposition products of sulphathiazole. This is the fourth report in serial investigations of the use of HPLC in the study of stability of selected sulphonamides. With their method, 2-Aminotriazole and sulphanilic acid were determined in a solution of sulphathiazole in 1M-HCl maintained at elevated temperature .

Bergh and Breytenbach[44] analyzed trimethoprim in pharmaceuticals by a developed HPLC method. The cited drug and five of its degradation products can be separated using this procedure. Sulphamethoxazole, methyl 4-hydroxybenzoate and propyl 4-hydroxybenzoate, usually present in such formulations, did not interfere.

The simultaneous determination of cefapirin, cefapirin lactone and desacetylcefapirin in sodium cefapirin bulk and injectable formulations was reported by MacNeil et al.[45].

The stability of an aqueous solution of mezlocillin sodium with phosphate buffer solution or other intravenous admixture ingredients such as dextrose, fructose and NaCl determined by HPLC was described by Das-Gupta[46]. The optimum stability was observed at pH 4.8; solution containing dextrose (5%) and NaCl (0.9%) were stable for up to 4 days at 25°C, 36 days at 5 °C, and 60 days at -10°C.

The stability of sissomicin in hydrophilic petrolatum ointment was investigated by Yamamura et al.[47]. They found little degradation in ointment stored at 5°C in the dark for 90 days.

Lauback et al.[48] determined ampicillin in bulk, injectables, capsules and oral suspensions by reversed-phase ion-pair HPLC. This rapid, precise, stability-indicating assay was specific for ampicillin in the presence of phenylglycine, phenoxymethylpenicillin, 6-aminopenicillanic acid, penicilloic acid and the analogous acid derived from ampicillin. Roy et al.[50] studied the stability of locally manufactured ampicillin trihydrate by HPLC with gradient elution. Residual solvents were determined in samples of ampicillin trihydrate by headspace GC with FID; methanol, 2-propanol, CH_2Cl_2, 2-methyl-1-propanol and triethylamine were found.

Grobben-Verpoorten et al.[49] determined the stability of tetracycline suspensions by HPLC. The influence of pH, buffer and temperature and time of storage was examined. They found the most important parameter affecting stability to be pH; pH 4 to 5.5 at room temperature yielded suspensions stable for $\geqq$3 months.

Cephradine dosage forms were analyzed by Das-Gupta[51]. The effects of pH on the decomposition of the drug were studied.

The application of a new method of freeze-drying for the determination of amoxycillin in pharmaceutical formulations was investigated by Tico-Grau et al. [52]. The method is suitable for stability studies and was used to separate the drug, the corresponding penicilloic acid and polymeric forms of amoxycillin.

An HPLC method utilizing either ultraviolet or electrochemical detection was used for the determination of erythromycin estolate in pharmaceutical dosage forms and was applied to the study of dosage form content uniformity before bioavailability assessment by Stubbs and Kanfer [53]. Special considerations related to the stability of the estolate moiety during sample manipulation and storage in the autosampler are discussed. Use of the electrochemical detector was found to be advantageous in stability studies.

Parasrampuria and Das-Gupta [54] quantitated cefadroxil in pharmaceutical dosage forms. A variety of excipients did not interfere. The procedure was used to determine the stability of suspensions after formulation and storage at 5°C.

Lorenz et al. [55] determined 19 process-related impurities and degradation products such as Δ-2-cefaclor, phenylglycine and isocephalexin in cefaclor by HPLC. Stability studies revealed that the concentration of the degradation products increased by approximately 0.6% per year.

Rogstad and Weng [56] extracted and analyzed oxytetracycline, flumequine and oxolinic acid in a drug delivery system for farmed fish. Recoveries of all three antibiotics were quantitative. The method was used in stability studies of oxytetracycline.

YTR 830H (tazobactam) and piperacillin in pharmaceutical preparations were analyzed by Tsukamoto and Ushio [57]. The within- and between-day RSD were 0.45% and 0.78%, respectively, for tazobactam and 0.33% and 0.52%, respectively, for piperacillin. The method was applied to stability studies.

A stability-indicating HPLC assay method for the determination of a new distamycin derivative, N-4-[NN-Bis-(2-chloroethyl)amino]benzoyl-N-deformyldistamycin A (FCE 24517), with antitumoural activity was developed by Panizza and Farina [58]. The method was used for determination of the drug in samples degraded under acidic and basic conditions, in the presence of an oxidizing agent and under intense light in order to evaluate stability and shelf-life. The drug was well separated from degradation products and from synthesis impurities.

Cephalosporin and its primary degradation product were separated and determined by Hayward et al. [59]. The HPLC method was applied in stability testing.

Stiles et al. [60] determined the stability of cefuroxime when prefilled in Pharmacia Deltec CADD-PLUS drug reservoirs and subjected to various conditions including: (1) a 24-hour infusion cycle at near-body temperature (30°C), (2) a 7-day refrigerated (3°C) storage, and (3) a 30-day frozen (-20°C) storage followed by 4 days of refrigeration prior to infusion at 30°C, by a stability-indicating HPLC assay.

Das-Gupta and Parasrampuria [61] developed a simple stability-indicating HPLC method for the quantitative determination of cephalexin in pharmaceutical dosage forms. The method was applied in stability studies of cephalexin. Excipients in the dosage forms did not interfere.

IV. *Antidiabetic Agents*

The quantitation of chlorpropamide and tolbutamide in tablets was developed by Das-Gupta [62]. There was no interference from the products of hydrolysis or from excipients in tablets. The products of hydrolysis (4-chlorobenzenesulphonamide and toluene-p-sulphonamide) could be quantified if required.

V. *Antiepileptics*

An ion-pair HPLC procedure for the determination of nafimidone [1-(2-naphthoylmethyl)imidazole hydrochloride and its decomposition products was described by Taylor et al. [63]. Use of 85mM-octanesulphonic acid in mobile phase allowed the separation of the acidic decomposition products from the cited drug (but the thermal decomposition product was highly retained). The system with 2mM-tetrabutylammonium bromide, however, was found to be capable of separating all the species.

VI. *Antifungal Agents*

Christinat and Zulliger [64] developed a stability-indicating HPLC method for the determination of topical preparations of econazole nitrate (Pevaryl) and Pevaryl with triamcinolone acetonide (Previsone) in cream and lotion formulations. Separation of two possible degradation products of Pevaryl from it and the internal standard (miconazole nitrate) was achieved.

Bakker et al. [65] described a protocol for quality control and stability testing of cytarabine and its injectable formulations, e.g. , Cytosar. An HPLC procedure was used for the quantitation. Determination of the drug after heating at 60°C, 70°C or 85°C for several days showed a decline of cytarabine concentration in parallel with loss of optical activity. The calculated shelf life of cytarabine at 25°C was 4.9 years, and that of the injectable form was $\geqq$ 2 years at room temperature .

VII. *Antihistamines*

Lo and Krause [66] developed a reversed-phase HPLC procedure for the simultaneous determination of probenecid and colchicine in solid dosage form. Tablets were heated at 60°C for 7 days with moisture to study the possible thermal-degradation. The method was found useful for routine quality control and stability studies.

Yuan and Locke [67] investigated the stability of diphenhydramine in liquid and solid drug preparations at temperature ranging from 42°C to 62°C over a period of 16 weeks using HPLC. It was found that the solid product is more stable than the liquid product and the diphenhydramine is well separated from any interferring peaks.

VIII. *Antihypertensive Agents*

Pharmaceutical dosage forms containing hydralazine hydrochloride and phenylpropanolamine or hydrochlorothiazide (as internal standard) were determined on two columns (30 cm×3.9 mm) packed with semipolar μ-phenyl or non-polar μ-C18 by Das-Gupta [68]. The method is stability-indicating since the product(s) of decomposition canbe separated from the intact drug. The μ-C18 column was recommended for stability studies.

Prazosin and polythiazide in the diuretic-antihypertensive combination products were simultaneously determined by Bachman [69] using HPLC. The described method can separate each drug from its impurities and can be used for stability-indicating assay of both drug substances and their impurities.

IX. *Antimuscarinic Agents*

HPLC was used to study the dissociation constant, partition coefficient, solubility and stability of oxybutynin in vitro by Miyamoto et al. [70]. Oxybutynin was found to be very stable in buffer of pH 1-8, but degraded rapidly at alkaline pH. Degradation half-life was 14 min. at pH 12.

Selkirk et al. [71] determined orphenadrine hydrochloride and its major degradation product o-methylbenzhydrol in syrup by HPLC. Degradation of orphenadrine hydrochloride after 99 days' storage was 0.06% at room temperature and 1.05% at 50°C.

Selkirk et al. [72] determined orphenadrine hydrochloride and its metabolite 2-methylbenzhydrol in dosage forms by reversed-phase HPLC.

X. *Antimycobacterial Agents*

Cendrowska et al. [73] analyzed 5-aminosalicylic acid and salicylic acid in tablets and suppositories using an HPLC method which was used in a stability study. It was found that 1% decomposition of 5-aminosalicylic acid occurred at room temperature over 1 year.

XI. *Antineoplastic Agents and Immunosuppressants*

Some pharmaceutically important nitro-compounds, furazolidone, nitrofurazone, nitrofurantoin, niridazole and nifuroxime in pharmaceutical

preparations were analyzed by Hassan et al. [74]. It was found that exposure to sunlight, the drugs decomposed to 5-nitrofuraldehyde, detected at 311 nm, so the method may be used in stability studies.

Fleming and Stewart [75] simultaneously determined cisplatin and 5-fluorouracil in 0.9% sodium chloride for injection. Within-day coefficients of variation (n = 10) for the cited drugs were 1.1 and 3.2%, respectively; corresponding between-day coefficients of variation (n = 50) were 2.1 and 3.7%.

Northcott et al. [76] investigated the determination of ormaplatin by LC. The method was used to establish the stability of ormaplatin in H_2O.

Guchelaar et al. [77] studied the stability of 1,2-diaminomethyl-cyclobutane-platinum(II)-lactate (lobaplatin; D19466), the new anti-cancer platinum analogue, in intravenous solutions using an HPLC procedure which was employed to characterize the stability of lobaplatin in infusion media.

Aqueous solution of carboplatin were transferred to the various containers containing 5% glucose solution and the containers were maintained at 25, 40, 50 and 60°C, protected from light. At specific time intervals samples were removed and analyzed by Prat et al. [78]. It was found that all decomposition product peaks were well resolved from the peak of carboplatin. Results showed that the degradation of carboplatin followed pseudo-first-order kinetics and no dependence on the nature of the container was found. A simultaneous HPLC analysis of carboplatin and cisplatin in infusion fluids was described by Rochard et al. [79]. The method was applicable in a stability-indicating assay; cisplatin was detectable only in acid solution.

Law and Jang [80] determined mitoxantrone in liposome preparations using HPLC and solid-phase extraction. Within-day and between-day RSD (n =10) were 0.51 and 1.22%, respectively. The method was used for the determinatin of the stability of mitoxantrone in liposome preparations.

Dietrich et al. [81] separated cytarabine and its decomposition products, 1-β-D-arabinofuranosyluracil, cytosine, uracil and cytidine using HPLC on a special reversed-phase packing (EnCaPharm 100 RP18, 5 μ m). The method was especially suited to stability studies of solution of the cited drug.

Vials containing 50 or 500 μ g of a new anthracycline analogue, 3'-deamino-3'-[2(S)-methoxy-4-morpholino]doxorubicin (FCE 23762) in the final drug formulation were analyzed by Rossini and Farina [82]. The method allows the determination of the cited drug in samples forcibly degraded under acidic and basic conditions, under intense white light and in the presence of a strong oxidizing agent, without interference arising from the side-products formed.

Chlorambucil and its N-oxide prodrug, chlorambucil N-oxide were determined by Chandler et al. [83] using HPLC. The stability of chlorambucil and chlorambucil N-oxide in buffer solution and alcohols, and their stability during the processing was established. Chlorambucil was found to decompose with time in 0.1M Tris (pH 7.5), but its prodrug was stable in Tris.

The separation of major ipecacuanha alkaloids, emetine and cephaeline, in linctus and pastille preparations were performed by Elvidge et al. [84] using HPLC. Degradation products yielded no interference with emetine and cephaeline.

Clark et al. [85] analyzed dibromodulcitol [mitolactol] in aqueous solution using HPLC. Complete separation of dibromodulcitol [mitolactol] from its degradation products was achieved.

Chow et al. [86] determined etoposide under various conditions of pH using HPLC with a reversed-phase octyl column. For degradation studies, the etoposide solution was boiled for 3 min. in concentrated HCl and in 0.1M-NaOH before chromatography. Baseline separation was observed. Floor et al. [87] sequentially quantitated the antineoplastic agent etoposide and benzyl alcohol, as well as impurities and degradation products in injectable formulations. Determination was based on peak-area ratios, and

the technique allowed the assay of etoposide, benzyl alcohol, benzaldehyde (a potential decomposition product) induced by acids, bases, heat and light, and all the major impurities from the synthesis reaction. Impurity levels as low as 0.2% were detectable by the method.

XII. *Antiviral Agents*

Pramar et al. [88] reported a stability-indicating HPLC method for the determination of acyclovir in capsules, ointment and injection formulations. Recovery was quantitative and no interference was observed from excipients. The drug was found to be more stable in alkaline than in acidic solution.

Shah et al. [89] determined ribavirin in capsule and syrup. Related impurities triazole carboxylic acid, ribose triazole carboxylic acid and triazole carboxamide were also determined by the same method. A comparative study of the advantages of the proposed method over the USP method is included.

A stability-indicating, gradient-elution HPLC assay of zidovudine in syrup was developed by Radwan [90]. Zidovudine was dissolved (100 mg/10 mL) in U. S. P. XXII syrup and stored in amber glass vials at 50 and 25°C for 2 and 17 weeks, respectively, or in colorless glass vials at 25°C for 2.5 weeks. Then the HPLC method was applied for the analysis of the drug in commercial preparations.

XIII. *Anxiolytic Sedatives Hypnotics and Neuroleptics*

Panaggio and Greene [91] studied the stability of haloperidol using HPLC. It was found that haloperidol samples stored at 25°C were stable for at least 14 days except when exposed to light. Extensive degradation of this drug was noted at temperature above 60°C and at high pH.

Beaulieu and Lovering [92] determined perphenazine and its sulphoxide in liquid formulations. It was reported that the calibration graph was rectilinear for 5 to 108 ng of perphenazine sulphoxide and for 0.5 to 1.4 μ g of perphenazine.

Diazepam and its degradation products were determined by Hewala [93] using HPLC and GLC. and applied to a stability study.

The racemate of psychotropic drug levomepromazine (methotrimeprazine) was separated by Sztruhar et al. [94] using a chiral stationary phase. Effects of mobile phase composition and flow rate, pH and temperature were discussed. The calibration graph was rectilinear from 0.5 to 10% of R-(+)-enantiomer impurity with a detection limit of approximately 0.1%. The method could be applied to stability trials and enantiospecific bioavailability studies of pharmaceutical dosage forms.

An HPLC assay for oxazepam tablets and capsules was developed by Reif and DeAngelis [95]. By this technique oxazepam was separated from all known degradation products. Degradation products were isolated by t. l. c. and identified to validate the specificity of the method.

Bounine et al. [96] determined of zopiclone in tablets. Zopiclone and its potential degradation products were separated and simultaneously determined in ~15 min. with detection limits of 0.05-0.2%. Recoveries were ~100% with RSD of 0.23-0.48%.

(To be continued in next issue)

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Table 1. Stability-indicating HPLC methods for drugs in pharmaceutical dosage forms

Drug	Materials	Columns	Mobile phases	Internal standards	Detection
ketoprofen Ref. 17	capsules, injections	Zorbax ODS C18, 15 cm×4.6 mm	0.05M KH_2PO_4 (adjusted to pH 2.5 with glacial acetic acid) - acetonitirle (55/45, v/v), 1.2 ml/min	flurbiprofen	265 nm
carprofen Ref. 18	tablets	Chromegabond C-18, 10 μm	methanol - 1% acetic acid (65:35)	benzophenone	254 nm
dichlorophen Ref. 19	capsules	μ-Bondapak C18, 30 cm×3.9 mm	methanol - water (3:1), 1.5 ml/min		290 nm
procarbazine hydrochloride Ref. 20	capsules	Partisil PXS 10/25 ODS-2, 10 μm, 25 cm	aq. 44% methanol containing 0.05M ammonium phosphate, pH 5.5	cinnamyl alcohol	254 nm
chlorpromazine Ref. 21	tablets injections	LiChrosorb-NH2, 5 μm, 25 cm×4 mm containing 0.01% each of	acetonitrile - 0.1mM-HCl (pH 4.00; 83:17), $Na_2S_2O_5$ and isoascorbic acid and 0.06% of NaCl, 1 ml/min	quinine hydrochloride, or cinchocaine hydrochloride for sulphone and sulphoxide	excitation at 280 nm, fluorimetric detection at 450 nm, and 385 nm for sulphoxide and sulphone
diclofenac sodium Ref. 22	raw material, pharmaceutical, solid dosage forms	Ultrasphere IP, 5 μm 15 cm×4.6 mm	acetonitrile/methanol/ pic B-6 buffer (1:1:2), 2-3 ml/min	diflorasone diacetate	229 nm
gabexate mesylate Ref. 23	gel ointments	μ-Bondapak C18, 30 cm×3.9 mm	aq. 70% methanol containing 5mM-Na pentane-1-sulphonate		235 nm
prostaglandins Ref. 23	gel ointments	μ-Bondapak C18, 30 cm×3.9 mm	0.02M-KH_2PO_4 - acetonitrile (3:2)		214 nm
prednisolone Ref. 23	gel ointments	μ-Bondapak C18, 30 cm×3.9 mm	aq. 60% methanol		254 nm
butyl flufenamate Ref. 23	gel ointments	μ-Bondapak C18, 30 cm×3.9 mm	methanol		280 nm
indomethacin Ref. 24	controlled-release suppositories	Zorbax-CN, 25 cm×4.6 mm	methanol - 0.05% acetic acid (9:11), 1.2 ml/min	1,3-dinitrobenzene	254 nm
sulindac Ref. 25	tablets	Ultrasphere XL ODS, 3 μm, 7 cm×4.6 mm	50mM-ammonium acetate buffer (pH 6.0) - methanol (1:1), 1 ml/min	propyl paraben	280 nm
tebufelone Ref. 26	bulk, capsules or feed mixtures	25 cm×4.6 mm, Zorbax ODS, 5 μm; pre-column: RP-18, 3 cm×4.6 mm	aq. 70% acetonitrile containing 0.01% of K_3PO_4, pH 3.5, 2 ml/min	2,6-di-t-butyl-4-methylphenol	280 nm
nefopam hydrochloride Ref. 27	bulk	NOVA PAK C18, 5 μm 15 cm×3.9 mm	acetonitrile - 0.2M-phosphate buffer (1:3; pH 3.03) containing 0.05M-1-pentane sulphonic acid sodium salt, 2.3 ml/min		201 nm
oxyphenbutazone	ointments	LiChrosorb RP-18,	0.1M-Tris citrate buffer		239 nm

Ref. 28	tablets and capsules	7 μ m, stainless-steel cartridge (25 cm×4 mm)	(pH 5.25) - acetonitrile - tetrahydrofuran (65:29:6), 1.3 ml/min; or the same system without tetrahydrofuran (9:11), 1.8 ml/min		
verapamil hydrochloride Ref. 29	tablets	μ -Bondapak Phenyl, 30 cm×4 mm	0.02M-KH2PO4 - acetonitrile (31:19), 2.4 ml/min	dextromethorphan hydrobromide	278 nm
verapamil hydrochloride Ref. 29	tablets	μ -Bondapak C18, 30 cm×4 mm	49% (v/v) methanol - 0.45% (v/v) anhyd. acetic acid - aq. 36mM-ammonium formate, 2.0 ml/min,		
cloxacillin Ref. 30	bulk capsules injections syrups	μ -Bondapak C18, 30 cm×3.9 mm	methanol - 4% acetic acid (3:2)	dimethyl phthalate	254 nm
dicloxacillin Ref. 31	bulk capsules	μ -Bondapak C18, 5 μ m 30 cm × 3.9 mm	methanol - 4% acetic acid (6:4), 1.5 ml/min	dimethylphthalate	254 nm
bacitracin A, B1, B2 and F Ref. 32	pharmaceuticals	Nucleosil 5 C18, 5 μ m or ChromSpher C18, 5 μ m, 15 cm×4.6 mm	50mM-KH_2PO_4 - (methanol - acetonitrile; 1:1) (51:49 or 27:23, respectively), 1.4 ml/min		220 nm
cephamandole and its nafate Ref. 33	infusion	C18 Interchim, 5 μ m 10 cm×4.6 mm	0.2% triethylamine of pH 2.5/acetonitrile (19:6), 1.5 ml/min		254 nm
benzoyl metronidazole Ref. 34	liquid preparations	Micropak MCH-10, 30 cm×4 mm	20mM-ammonium acetate in aq. 47% acetonitrile, adjusted to pH 4.5, 2 ml/min	ethyl 4-hydroxy-benzoate	270 nm
norfloxacin Ref. 35	aqueous solution	ODS Hypersil, 5 μ m 10 cm×2 mm	aq. 15% acetonitrile containing 10mM-disodium hydrogen phosphate, 2.5mM-sodium lauryl sulphate and 10mM-tetrabutylammonium bromide, 0.5 ml/min		fluorimetric detection at 418 nm (excitation at 280 nm)
norfloxacin Ref. 36	tablets	styrene-divinylbenzene reversed-phase materia 8 μ m, 25 cm×4.6 mm	aq. 8% acetic acid - acetonitrile - methanol - THF (1720:228:39:13), 1.8 ml/min		fluorescence detection at 440 nm (excitation at 285 nm)
norfloxacin glutamate, norfloxacin glucuronate Ref. 37	tablets and liquid	Hypersil ODS, 5 μ m 10 cm×4.6 mm	methanol/H_2O/diethylamine (125:125:1) adjusted to pH 5.5 with H_3PO_4, 1 ml/min	chloronitrodiazepine	278 nm
cefoperazone sodium Ref. 38	10% glucose soln. 0.9% NaCl soln. of Ringer soln.	Nucleosil-C18, 7 μ m operated at 37°C, 20 cm×4 mm	methanol - 10mM - KH_2PO_4 (9:11), 1 ml/min		254 nm
cephazolin Ref. 39	pharmaceuticals	LiChrosorb RP-18	0.01M-$(NH_4)_2HPO_4$ - methanol (3:1), 0.7 ml/min	cefoperazone	254 nm

tetryzoline hydrochloride Ref. 40	nasal sprays	RSIL C18, 15 cm ×4.1 mm	aq. 40% methanol containing 20mM-Na-octane-1-sulphonate and 10mM-NN-dimethyloctylamine, pH was adjusted to 3.0 with H_3PO_4, 1 ml/min	tolazoline hydrochloride	220 nm
cefuroxime sodium Ref. 41		Spherisorb hexyl, 5 μm 10 cm×4.5 mm	acetonitrile/acetate buffer of pH 3.4 (7:93)		diode-array detection at 273 nm
ceftazidime arginine Ref. 42	aqueous solution	Ultrasphere ODS, 5 μm 25 cm×4.6 mm	0.01M-amm onium acetate buffer - methanol (89:11), 1.5 ml/min		254 nm
sulphathiazole Ref. 43		Pragosil 5	1,2-dichloroethane - propan-2-ol - aq. 25% NH_3 - methanol (13:7:1:3), 110 ml/h		226 nm
trimethoprim Ref. 44	tablets	Zorbax TMS, 7- μm 25 cm×4.6 mm	H_2O - THF - propanol - methanol - acetonitrile - acetic acid (34:25:20: 15:5:1), 2 ml/min		271 nm
cefapirin Ref. 45	bulk and injectable formulations	μ-Bondapak C18 30 cm×3.9 mm	5% DMF - 0.2% acetic acid - 0.1% KOH soln. (45% w/w) in H_2O, 2 ml/min	acetanilide	254 nm
mezlocillin sodium Ref. 46		μ-Bondapak Phenyl 30 cm×4 mm	0.02M-ammonium acetate - aq. 42% methanol, 2 ml/min		230 nm
sissomicin Ref. 47	ointment	μ-Bondapak C18 30 cm×3.9 mm	2% Na_2SO_4 - 5mM - Na pentane-1-sulphonate, 1 ml/min		214 nm
ampicillin Ref. 48	bulk injectables capsules and oral suspensions	μ-Bondapak C18 30 cm×3.9 mm	solvent A (35mm -Na dodecyl sulphate - 2M-formic acid):acetonitrile: H_2O = 100:350:400, 3.0 ml/min		254 nm
tetracycline Ref. 49	suspensions	μ-Bondapak Phenyl 25 cm×4.6 mm	gradient elution, effected with methanol - water - 1M-H_3PO_4	4-hydroxybenzoic acid	280 nm
ampicillin trihydrate Ref. 50		Waters Radial Pak 10 C18, 10 cm×5 mm	gradient elution, pH 5.5 buffer - acetonitrile [89:11 (held for 7 min) to 79:21], 1.5 ml/min		230 nm
cephradine Ref. 51	pharmaceuticals	μ-Bondapak phenyl 30 cm×3.9 mm	6% of acetonitrile, 8% of methanol, 0.1% of anhyd. acetic acid and 0.01M-ammonium acetate, 2 ml/min	cephazolin	260 nm
amoxycillin Ref. 52	pharmaceuticals	Bondapak C18, 10 μm 30 cm×3.9 mm	0.02M-potassium phosphate buffer of pH 4.0 to 4.5 - methanol (3:17), 1.2 ml/min	D(-)-4-Hydroxy-phenylglycine	254 nm

erythromycin estolate Ref. 53	capsules suspensions tablets	C18, 10 μ m, at 35°C, 25 cm×3.9 mm stainless-steel	acetonitrile - 0.05M-phosphate buffer (pH 6.3) (13:7), 1.3 ml/min	oleandomycin phosphate	200 nm or dual-electrode ECD
cefadroxil Ref. 54	tablets capsules suspension	Microbondapak C18 30 cm×3.9 mm	2% acetonitrile in aq. 0.01M-ammonium acetate buffer, 2.0 ml/min	ceftazidime	254 nm
cefaclor Ref. 55		YMC ODS, 5 μ m 25 cm×4.6 mm	gradient elution, 0.69% NaH_2PO_4 adjusted to pH 4 with phosphoric acid - acetonitrile		220 nm
oxytetracycline Ref. 56		Cyano Spheri-5 MPLC, 10 cm×4.6 mm; similar guard column (3 cm×4.6 mm)	0.02M-oxalic acid - methanol (19:1) containing DMF (50 ml/l), 1 ml/min	demeclocycline hydrochloride	350 nm
flumequine Ref. 56		PLRP-S, 5 μ m, 15 cm×4.6 mm; similar guard column (1 cm×4.6 mm)	0.02M-H_3PO_4 - acetonitrile - THF (13:4:3), 0.7 ml/min	oxolinic acid	336 nm
tazobactam and piperacillin Ref. 57	pharmaceuticals	Ultron ODS-X, 5 μ m 15 cm×4.6 mm	10mM -tetra-n-butyl-ammonium hydroxide/5mM-K_2SO_4, acetonitrile and methanol (40:12:1), (pH 4.1; 0.7 ml/min)	methyl benzoate	220 nm and 270 nm
distamycin Ref. 58		Zorbax TMS (C1), 6 μ m 25 cm×4.6 mm	45% acetonitrile containing Na dodecyl sulphate (0.5 g/l), pH was adjusted to 2 with 2M-HCl, 1.4 ml/min		314nm
cephalosporin Ref. 59	injection soln.	Whatman 10 SAX, 10 μ m 25 cm×4.6 mm	acetonitrile - 0.15M NaH_2PO_4 (1:9), 1.5 ml/min		230 nm
cefuroxime Ref. 60	CADD-PLUS drug reservoirs	Nova-Pak C18	6% acetonitrile, 1% glacial acetic acid and water adjusted to a pH of 4.0, 1 ml/min		
cephalexin Ref. 61	pharmaceuticals	μ -Bondapak Phenyl, 30 cm×3.9 mm	9% of acetonitrile in aq. 0.01M-amm onium acetate, 2 ml/min		260 nm
tolbutamide Ref. 62	tablets	semi-polar μ -Bondapak Phenyl, 30 cm×4 mm	aq. mobile phase containing 45% (v/v) methanol, 0.02M-ammonium acetate and 0.5% (v/v) anhyd. acetic acid, 2.0 ml/min or 2.5 ml/min		232 or 254 nm
nafimidone Ref. 63		Hypersil ODS, 5 μ m 10 cm×2 mm	2mM-tetrabutylammonium bromide or 85mM-octane-sulphonic acid in acetonitrile - 0.04M-phosphate buffer of pH 7.0 (1:3), 0.5 ml/min		
econazole nitrate Ref. 64	creams lotion	μ -Bondapak C18, 30 cm×4 mm	methanol - tetrahydrofuran - 0.1% ammonium carbonate soln. (39:1:10),	miconazole nitrate	220 nm

			2 to 2.3 ml/min		
cytarabine Ref. 65	injectable formulations	Nucleosil 5 C18, 25 cm×4.6 mm	KH_2PO_4 (13.6 g/l, pH 3.0), 1.5 ml/min	2-deoxyuridine	254 nm
probenecid, colchicine Ref. 66	tablets	C18 material, 12.4 cm×4.6 mm	methanol - phosphate buffer soln. of pH 3.0 (11:9), 1.2 ml/min		244 nm
diphenhydramine Ref. 67	tablets	LiChrosorb RP8, 10 μm 25 cm×4.6 mm	acetonitrile - 5mM-sodium hexanesulfonate - acetic acid (7:3:0.1), 1.8 ml/min		258 nm
hydralazine hydrochloride Ref. 68	pharmaceuticals	semipolar μ-phenyl or non-polar μ-C18, 30 cm×3.9 mm	15mM-KH_2PO_4 containing 0.1% of acetic acid and 0.5% of methanol (2% for μ-C18), 3 ml/min		256 nm
prazosin, polythiazide Ref. 69	capsules	IBM Cyano, 5 μm 25 cm×4.5 mm	0.05M-sodium phosphate buffer (pH 3) - acetonitrile (13:7), 1.7 ml/min	benzophenone	268 nm
oxybutynin Ref. 70		YMC-pack Protein-RP, 15 cm×4.6 mm	40% methanol/20mM-KH_2PO_4 adjusted to pH 3.5, 0.5 ml/min		220 nm
orphenadrine hydrochloride Ref. 71 and Ref. 72	syrup and dosage forms	ODS-Hypersil, 5 μm 10 cm×5 mm	acetonitrile - aq. 50mM-KH_2PO_4 (1:1) containing 40mM-Na dodecyl sulphate at pH 4.0, 2 ml/min	diphenhydramine hydrochloride	220 nm
5-aminosalicylic acid Ref. 73	tablets and suppositories	LiChrosorb RP-18, 10 μm, 25 cm×4 mm	22% acetonitrile containing 0.5% of acetic acid, 1 ml/min; or methanol - 50mM-NaH_2PO_4 - 25mM-Na_2HPO_4 - tetrabutylammonium phosphate (12:44:44:1), 0.6 ml/min		300 nm or 229 nm
furazolidone, nitrofurazone, nitrofurantoin, niridazole, nifuroxime Ref. 74	pharmaceuticals	LiChrosorb RP18, 25 cm×4 mm	isocratic methanol - H_2O - Britton - Robinson buffer (pH 3) (8:11:1), 1 ml/min		365, 375, 368 and 340 nm, respectively
cisplatin, 5-fluorouracil Ref. 75	injection soln.	Spherisorb ODS 1, 5 μm 15 cm×4.6 mm	2% tetrabutylammonium hydroxide, adjusted to pH 6.0 with H_3PO_4, 1 ml/min		313 nm
ormaplatin Ref. 76		Dynamax Microsorb, 5 μm, 25 cm×4.6 mm; guard column: 3 μm 3 cm×4.6 mm	H_2O		254 nm
lobaplatin Ref. 77	intravenous solutions	Supelcosil LC-1, 5 μm 25 cm×4.6 mm	0.1M-phosphate buffer of pH 7 - acetonitrile (9:1), 1 ml/min		230 nm
carboplatin	5% glucose	Spherisorb-Ph, 5 μm	2% methanol,		210 nm

Ref. 78	solution	25 cm×4.6 mm	0.8 ml/min		
carboplatin, cisplatin Ref. 79	infusion fluids	Nucleosil C18, 5 μm 15 cm×4.2 mm	0.01M-phosphate buffer of pH 7 containing 0.55mM aq. 0.5% hexadecyltrimethyl-ammonium bromide, 1 ml/min		216 nm
mitoxantrone Ref. 80	liposome preparations	LiChrospher RP-18 5 μm, 25×4.6 mm	0.01M-KH_2PO_4/acetonitrile (3:2) adjusted to pH 3.0 with H_3PO_4, 1 ml/min	Propyl paraben	242 nm
cytarabine Ref. 81		EnCaPharm 100 RP18, 5 μm, 12 cm×4.6 mm	phosphate buffer soln. of pH 7		260 nm
3' -deamino-3' -[2(S)-methoxy-4-morpholino]-doxorubicin Ref. 82	-	Zorbax Rx-C8, 5 μm 25 cm×4.6 mm	aq. 30% acetonitrile containing 2 ml/l of 85% H_3PO_4 (pH 6.0)		254 nm
chlorambucil, chlorambucil N-oxide Ref. 83	Tris buffer of pH 7.5	Nova-Pak C18, 4 μm 15 cm×2 mm	methanol/aq. trifluoro-acetic acid of pH 2.25 (13:12), 0.35 ml/min	3-(4-hydroxyphenyl) propionic acid	249 nm using a diode array spectrophotometer
emetine, cephaeline Ref. 84	pharmaceuticals	μ-Bondapak C18 15 cm×3.9 mm	methanol - H_3PO_4 - H_2O (400:1:599) containing 1 g/l of heptane-1-sulphonic acid, 2 ml/min	Ethyl 4-hydroxybenzoate	fluorescence detection at 304 nm (excitation at 276 nm
mitolactol Ref. 85	aq. soln.	Spherisorb ODS, 5 μm 30 cm	2% methanol		differential refractometric detection
etoposide Ref. 86	bulk	LiChrosorb RP-8, 5 μm 15 cm×4.6 mm	acetonitrile - acetic acid - H_2O (27:1:72; pH 4), 1.5 ml/min	methoxsalen	230 nm
etoposide benzyl alcohol Ref. 87	injectable formulations	μ-Bondapak Phenyl, 10 μm, 30 cm×39 mm	0.02M-Na acetate (pH 4) containing 26% of acetonitrile, or acetate buffer soln. containing 60% of acetonitrile	methyl 4-aminobenzoate or biphenyl	254 nm
acyclovir Ref. 88	capsules, ointment, injection soln.	μ-Bondapak C18, 30 cm×3.9 mm	3% of acetonitrile in 0.01M-KH_2PO_4 buffer soln., 2 ml/min	salicylic acid	252 nm
ribavirin Ref. 89	capsules, syrup	μ-Bondapak C18 30 cm×3.9 mm	0.01M-potassium dihydrogen phosphate/ methanol (19:1, pH 4.6), 1 ml/min		207 nm
zidovudine Ref. 90	syrup	μ-Bondapak C18, 10 μm, 30 cm×3.9 mm	gradient elution, acetonitrile/0.2% H_3PO_4 (1:9-4:6 in 10 min), 1.8 ml/min		264 nm
haloperidol Ref. 91	bulk	μ-Bondapak CN	40% tetrahydrofuran containing 0.75% of H_3PO_4		254 nm

perphenazine Ref. 92	tablets, liquid formulations	Zorbax CN, 5 μ m 15 cm×4.6 mm	25mM-acetate buffer (pH 4.5) - methanol - acetonitrile (3:3:4), 2.5 ml/min	trifluoperazine hydrochoride	229 nm
diazepam Ref. 93	pharmaceuticals	MOS-Hypersil	methanol - H_2O - acetic acid (80:20:1), 1.5 ml/min		255 nm
levomepromazine Ref. 94	pharmaceuticals	β -cyclodextrin bonded stationary phase, 5 μ m, 25 cm×4.6	methanol - aq. triethyl-ammonium acetate buffer of pH 4.5 (46:54), 1 ml/min		254 nm
oxazepam Ref. 95	tablets, capsules	Chromegabond C18, μ -Bondapak C18, Zorbax ODS or Partisil ODS-3, 25 to 30 cm×4.6 mm	methanol - H_2O - acetic acid (60:40:1), 2 ml/min		254 nm
zopiclone Ref. 96	tablets	LiChrospher-60 RP Select B, 5 μ m, 12.5 cm×4 mm	[18.06mM-monosodium hexane sulfonate/18mM-dihydrated monosodium dihydrogen phosphate (ion-pair buffer of pH 4.55)]/acetonitrile/ THF (81:18:1), 1.5 ml/min		303 nm