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Review article

An overview of the recent trends in development of HPLC methods for determination of impurities in drugs

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Abstract

An extensive survey of the literature published in various analytical and pharmaceutical chemistry related journals has been conducted and the high-performance liquid chromatography (HPLC) methods which were developed and used for determination of process-related impurities in drugs have been reviewed. This review covers the time period from 1995 to 2001 during which around 450 analytical methods including all types of chromatographic and hyphenated techniques were reported. HPLC with UV detection was found to be the technique of choice for many workers and more than 200 methods were developed using LC-UV alone. A critical analysis of the reported data has been carried out and the present state-of-art of HPLC for determination of impurities of analgesic, antibiotic, anti-viral, anti-hypertensive, anti-depressant, gastro-intestinal and anti-neoplastic agents has been discussed.

Keywords: Bulk drugs; Formulations; Impurities; HPLC

1. Introduction

Drugs play a vital role in the progress of human civilization by curing diseases. Today a majority of the drugs used are of synthetic origin. These are produced in bulk and used for their therapeutic effects in pharmaceutical formulations. There are biologically active chemical substances generally formulated into convenient dosage forms such as tablets, capsules, suspensions, ointments and injectables. These formulations deliver the drug substances in a stable, non-toxic and acceptable form, ensuring its bio-availability and therapeutic activity.

1.1. Quality, safety and efficacy of drugs

Safety and efficacy of pharmaceuticals are two fundamental issues of importance in drug therapy. The safety of a drug is determined by its pharmacological–toxicological profile as well as the adverse effects caused by the impurities in bulk and dosage forms. The impurities in drugs often possess unwanted pharmacological or toxicologi-

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cal effects by which any benefit from their administration may be outweighed [1]. Therefore, it is quite obvious that the products intended for human consumption must be characterized as completely as possible. The quality and safety of a drug is generally assured by monitoring and controlling the impurities effectively. Thus, the analytical activities concerning impurities in drugs are among the most important issues in modern pharmaceutical analysis. This has become quite evident by the recent publications of on this topic [2–5].

1.2. Origin of impurities

Impurities in drugs are originated from various sources and phases of the synthetic process and preparation of pharmaceutical dosage forms. A sharp difference between the process-related impurities and degradation products is always not possible. However, majority of the impurities are characteristic of the synthetic route of the manufacturing process. Since there are several possibilities of synthesizing a drug, it is possible that the same product of different sources may give rise to different impurities. For example, in some of the recent papers, the impurity profiles of orbofiban [6], trimethoprim [7], and fluxetine HCl [8] originated from different sources have been described. In these studies the impurity profiles of various lots of the bulk drugs obtained from different manufactures were compared. For example the high-performance liquid chromatography (HPLC) fingerprints of trimethoprim are shown in Fig. 1. It could be seen from Fig. 1 that the types of impurities vary from source to source. Generally, the origin of impurities could be from any of the following steps during synthesis.

- last intermediate of the synthesis
- products of incomplete reaction during the synthesis
- products of over reaction
- impurities in the starting materials of the synthesis
- impurities originating from the solvents of the reaction
- impurities originating from the catalysts
- products of side-reactions
- degradation products as impurities
- enantiomeric impurities
- residual solvents
- inorganic impurities
- impurities in excipients
- polymorphs as impurities

1.3. Types of impurities

Chemical impurities are classified as organic, inorganic and residual solvents for regulatory purposes [9]. Organic impurities can originate from impurities contained in starting materials (most often isomeric impurities), synthetic intermediates (incomplete reaction or excess reagent used) and degradation products which may depend on alterations in reaction conditions, such as temperature, pH, or in storage conditions (hydrolysis, oxidation, ring opening, etc.). Inorganic impurities may derive from the manufacturing process and are normally known and identified as reagents, ligands, inorganic salts, heavy metals, catalysts, filter aids and charcoal etc.

1.4. Regulatory aspects

Control is more important today than ever. Until the beginning of the 20th century, drug products were produced and sold having no imposed control. Quality was generally poor. Many products were patent medicines of dubious value. Some were harmful and addictive. In 1937, ethylene glycol was used as a vehicle for an elixir of sulfanilamide, which caused more than 100 deaths [10]. Thereupon the Food, Drug and Cosmetic act was revised requiring advance proof of safety and various other controls for new drugs. The impurities to be considered for new drugs are listed in regulatory documents of the Food and Drug Administration (FDA) [11], International Conference on the Harmonization of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) [12] and United States Pharmacopoeia (USP) [13]. Nevertheless, there are many drugs in existence, which have not been studied in such detail. The USP and National Formulary (NF) are the recognized standards for

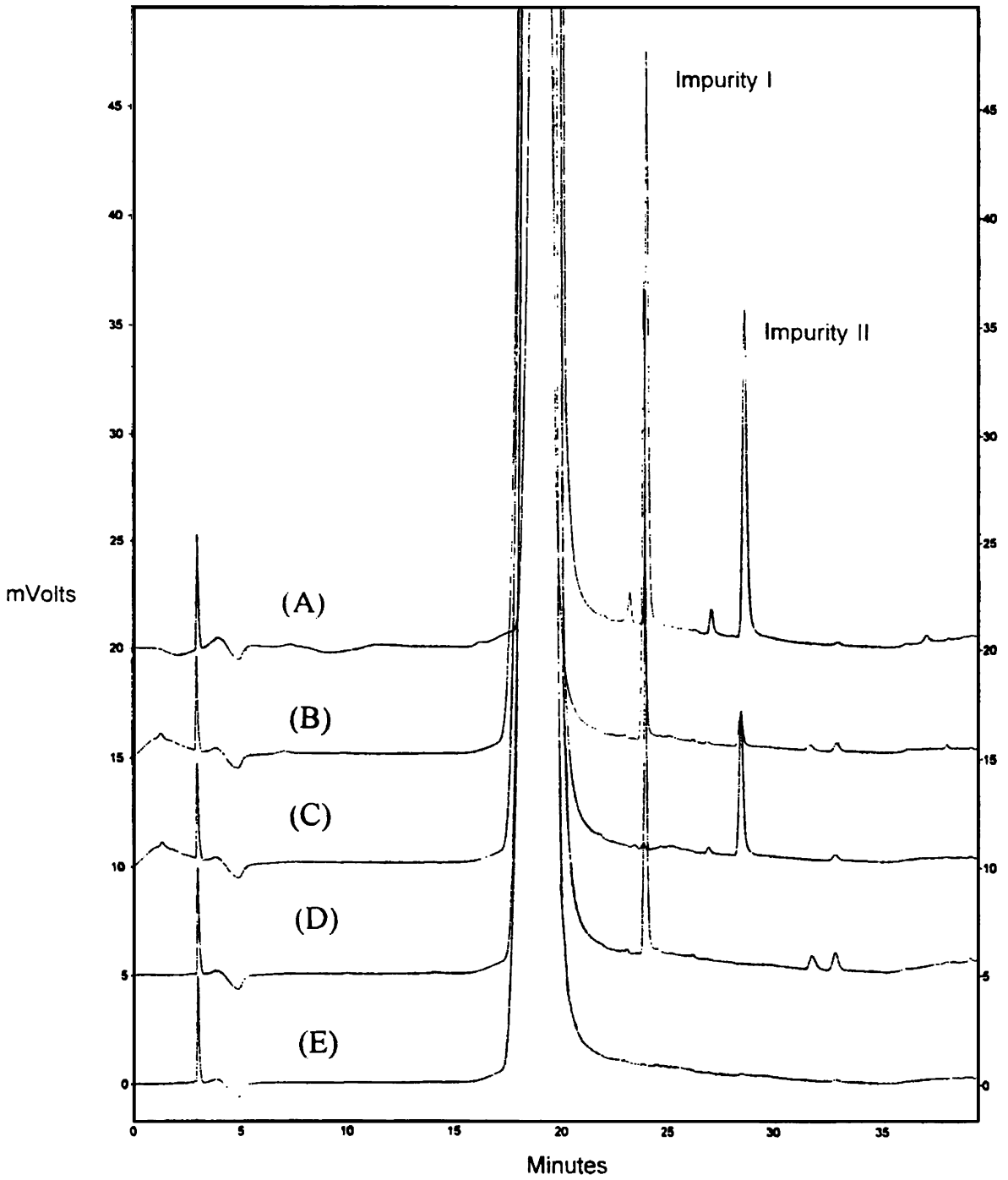


Fig. 1. HPLC profile of trimethoprim from different manufactures of China (A, B), Israel; (C) United States (D, E).

potency and purity of new drugs. These compendia have become official upon adoption of the first food and drug act. They formulate legal standards of quality, purity and strength of new drugs. The good manufacturing practices provide minimum quality standards for production of pharmaceuticals as well as their ingredients [14]. The ICH, which took place in Yokohama, Japan in 1995 has released new guidelines on impurities in new drug products [15]. These guidelines have a number of advantages, both for the industry and the regulators. The most critical aspect of the elaboration of the guidelines was the definition of the levels of impurities for identification and qualification (Table 1). Qualification is the process of acquiring and evaluating data for establishing the biological safety of an individual impurity or a given impurity profile at the levels specified. The level of any impurity present in a new drug substance that has been adequately tested in safety and clinical studies is considered qualified. A rationale for selecting impurity limits based on safety considerations has to be provided. The 'Decision Tree for Safety Studies' (Fig. 2) describes considerations for the qualification of impurities when thresholds are exceeded. This has some consequence for method development. Analytical procedures should be able to separate all the impurities from each other and the method should be optimized to separate and quantify them in the dosage forms. Such methods are to be validated demonstrating the accuracy, precision, specificity, limit of detection, quantification, linearity, range and interferences (Fig. 3). The validation of analytical procedures, i.e., the proof of its suitability for the intended purpose, is an important part of the registration application for a new drug [16,17]. Additional peak tailing, peak resolution and analyte recoveries are important in case of chromatographic methods. The ICH has harmonized the requirements in two guidelines [18,19]. The first one summarizes and defines the validation characteristics needed for various types of test procedures, the second one extends the previous text to include the experimental data required and some statistical interpretation. These guidelines serve as a basis worldwide both for regulatory authorities and industry and bring the importance of a proper validation to the attention of all those involved in the process of submission of drug master files. The analytical research and development units in the pharmaceutical industry are responsible for preparation and validation of test methods.

Table 1
ICH guidelines for identification and qualification of impurities in bulk drugs and formulations

Dose	Threshold for	
	Identification (%)	Qualification (%)
<1 mg	1.0	1.0
1–10 mg	0.5	1.0
10–100 mg	0.2	0.5
100 mg–2 g	0.1	0.2
>2 g	0.1	0.1

1.5. Monitoring of impurities in drugs

The monitoring of in-process impurities was an obscure and unidentified field about 20 years ago. Now it has become a major factor in modern pharmaceutical industry. This is mainly because of the pressure for product quality, and the demand for higher standards of process reliability. Toxicological issues have also brought about a greater sensitivity to the significance of impurities at trace levels [20]. New attention has been given to the various classes of toxicants present as impurities in pharmaceutical products. In view of these changes it has become necessary to pay more attention to the origins and pathways of a host of impurities within the process. Frequently, impurities are formed as isomers of the desired reaction products and a critical impurity can often enter with the feed. Analytical recognition of the problematic compounds is the first step towards the solution of the problem. Monitoring of the process reactions often lends valuable insight into the types of impurities that may be present. The purity of the final product may often be aided by controlling the purity of the materials used in its synthesis. Whenever possible, the levels of impurities originating for the starting materials should be limited through appropriate in-process controls in order

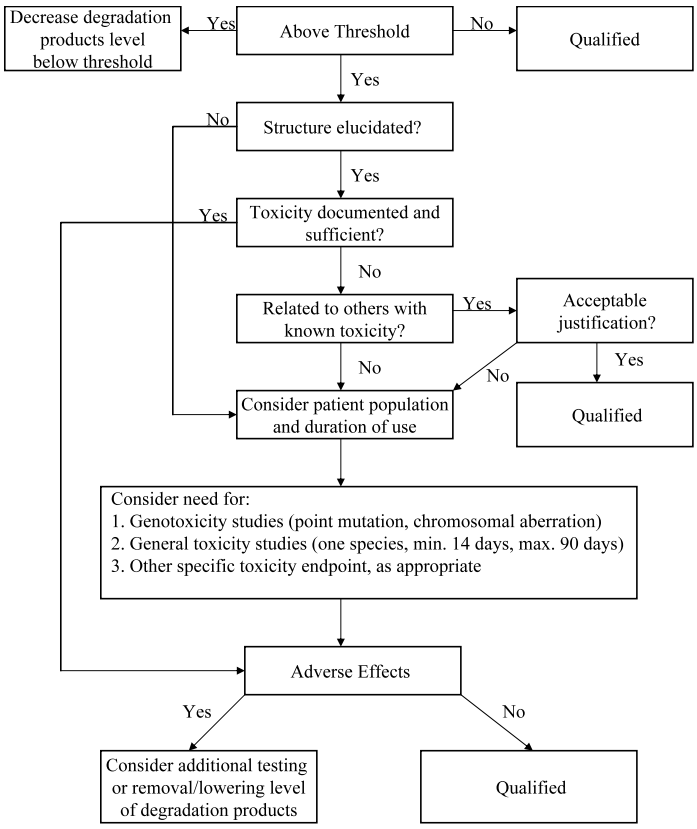


Fig. 2. ICH decision tree for safety studies.

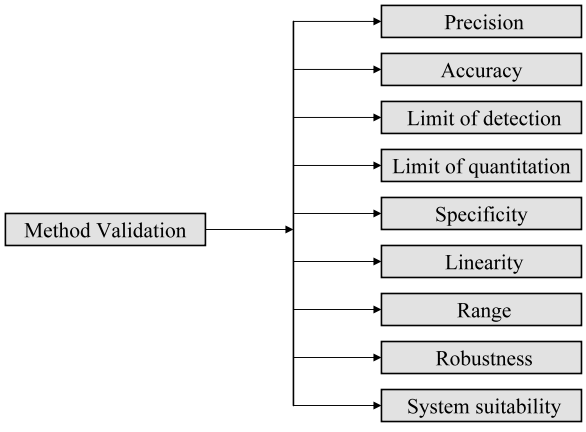


Fig. 3. ICH method validation parameters.

to avoid the need for their monitoring in the drug substance. The use of chromatographic techniques for monitoring the starting materials, intermediates, and the process reactions is an excellent means for controlling the purity of the final drug and thereby protecting the patient who ultimately receives it. The best way to characterize the quality of a bulk drug is to determine its purity. There are two possible approaches to reach this goal. The determination of the active ingredient content with a highly accurate and precise specific method or the determination of its impurities. In the early years of drug analysis, when chromatographic techniques were not yet available the characterization of the purity of drugs was based on the determination of the active ingredient content by non-specific titrimetric and photometric methods supported by the determination of physical constants and some limit tests for known impurities based mainly on colour reactions [21]. The deficiencies of this approach are well known. In many cases even highly contaminated drug materials

could meet the requirements set in the early editions of different pharmacopoeias. As a consequence of the enormous development of the analytical technology in the last two decades entirely new possibilities have been created for the determination of the purity of drug materials [22–24]. In principle, it is now possible to replace all non-specific assay methods with highly specific and precise (mainly HPLC) methods thus greatly improving the value of the determination of the active ingredient content of bulk materials. Nearly all organic impurities are determined by chromatographic or related methods of which HPLC has been the most important for over a decade. A thorough literature search has revealed that around 450 methods based on HPLC, capillary electrophoresis (CE), Gas–liquid chromatography (GLC), SFC, Thin-layer chromatography (TLC) etc. were published during the period 1995–2001 of review. Fig. 4 shows the pie diagram indicating the percent usage of each technique from which it becomes very clear that HPLC has been the main technique used for analysis of impurities in drugs. An average of 30 papers per year were published during the period of review. A bar diagram showing the pattern of publication of HPLC methods is shown in Fig. 5. Most workers used the reversed-phase mode with UV absorbance detection whenever appropriate, because this provided the best available reliability, analysis time, repeatability and sensitivity. In fact, this technique has set the standard against which others are compared (Fig. 6). When compared with other techniques, the chromatographic efficiency of

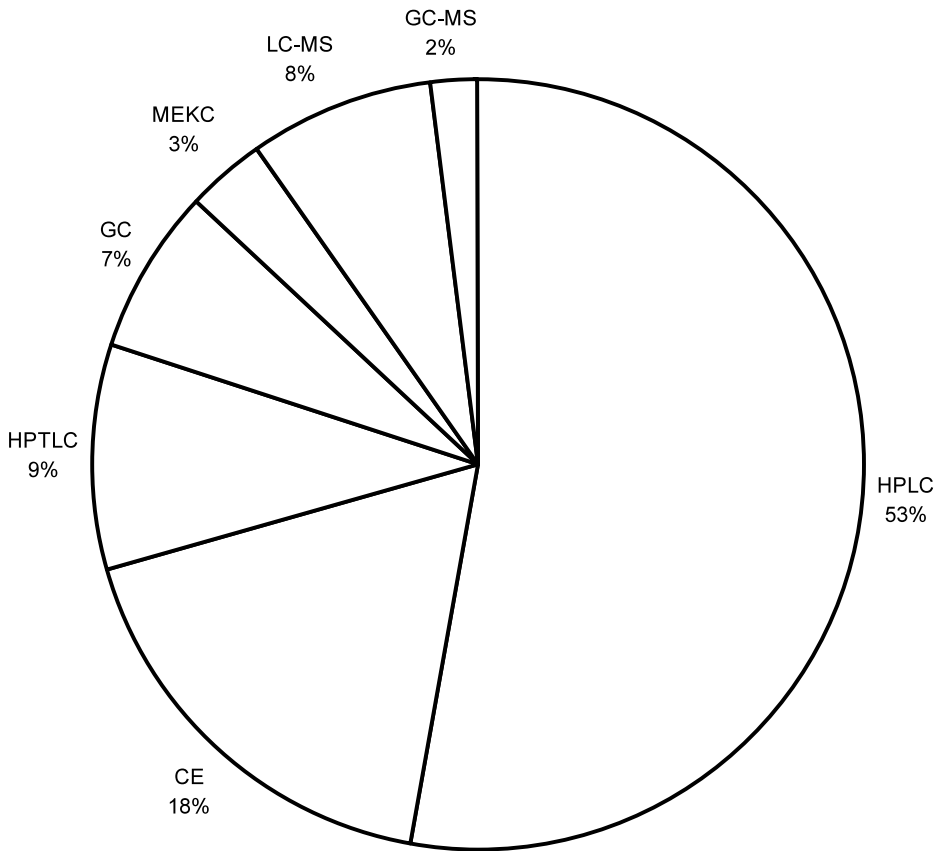


Fig. 4. Pattern of use different chromatographic techniques employed for analysis of impurities in drugs during 1995–2001.

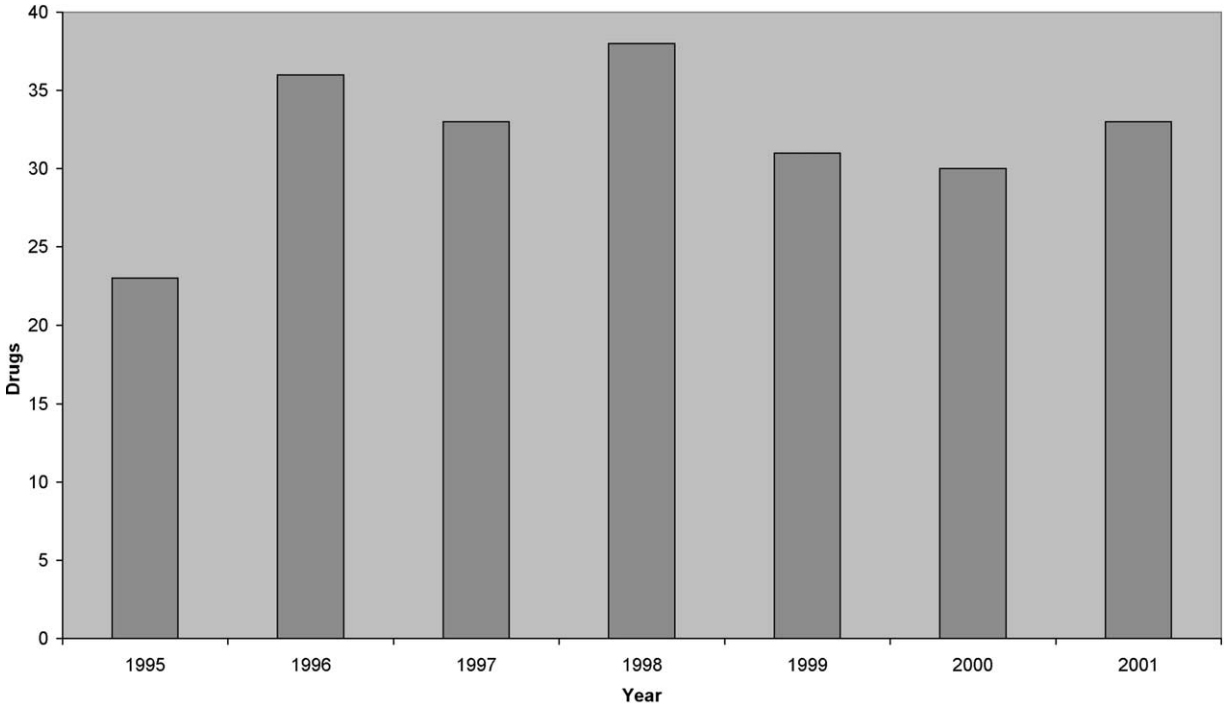


Fig. 5. Year-wise publication of HPLC methods appeared in the literature during 1995–2001.

HPLC appears modest, but this is compensated by the possibilities for varying retention and selectivity.

2. Role of chromatographic techniques in determination of impurities in drugs

TLC is a powerful tool for screening unknown materials in bulk drugs [25]. It provides a relatively high degree of assurance that all possible components of the drug are separated. However, its application to bulk drugs is limited owing to the problems involved in detection systems. GLC commands a significant role in the analysis of pharmaceutical products [26]. The advent of high-molecular-weight drug products such as polypeptides, or thermally unstable antibiotics limits the scope of this technique. Its principal limitation rests in the relative non-volatility of the drug substances. Therefore, derivatization is virtually mandatory, but the techniques for producing volatile derivatives of drugs are legion. New advances in the development of supercritical fluid chromatography and CE hold more promising in solving these problems [27–29]. At present, HPLC is the most widely used technique for the analysis of bulk drugs and their formulations [30]. Derivatization of the drugs prior to analysis is normally not required. The sample preparation is extremely simple and the errors associated with it are generally kept to a minimum by using HPLC. Gradient elution, temperature and wavelength-programming techniques provide valuable information regarding the undetected components of a given drug. Generally speaking, gradient elution, although extensively used in pharmaceutical research, is not popular because many of the above advantages are lost. Instead, screening for potential impurities is often performed by a combination of isocratic HPLC methods. For example the search for 11 potential impurities of mizolastine required the use of three isocratic HPLC methods because of the large differences in the hydrophobicities of the impurities [31]. The choice of proper detection mode is crucial to ensure that all the

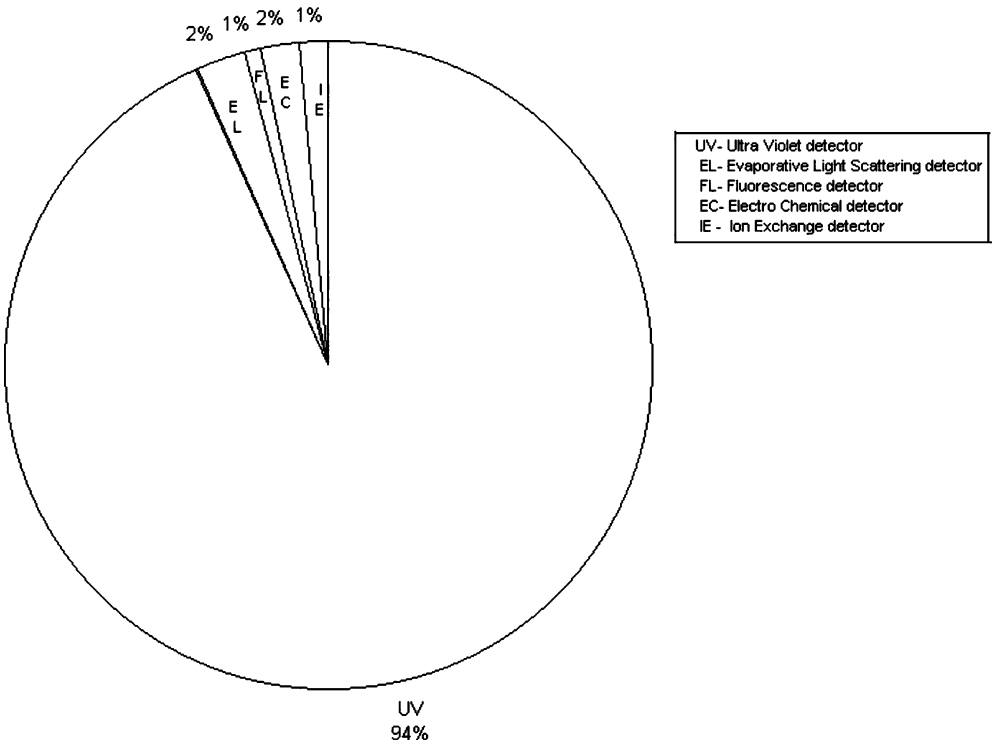


Fig. 6. Usage of different detectors for HPLC analysis of impurities in drugs.

components are detected. With UV detection, this problem could be overcome by using a multiple wavelength scanning program which is capable of monitoring several wavelengths simultaneously. It provides assurance that all the UV-absorbing components are detected, if present in sufficient quantity. Photodiode-array detectors are used to record spectro-chromatograms simultaneously. Fluorescence, electrochemical, refractive-index, and conductivity detectors are appropriate for specific applications. Chiral detectors are useful in determining the purity of enantiomeric drugs by HPLC. Several books and articles have dealt the theory and practices of HPLC [32,33]. Many research papers have described the latest developments in its instrumentation and applications in the pharmaceutical industry [34,35]. Thus, HPLC provides a major service in answering many questions posed by the pharma industry. However, the limitations of HPLC include the cost of columns, solvents and a lack of long-term reproducibility due to the proprietary nature of column packings. Moreover in case of absorbance detector, there exists no alternative having the same combination of performance characteristics. HPLC may, therefore, be complemented by GC, TLC or CE, and in some cases by tests not involving a separation method [36–38]. If a diode array detector is combined with a separating column, three dimensions i.e. three co-ordinates become available: wavelength, intensity and chromatographic retention [39]. A particular drug substance appears as a point in this three dimensional space. The spectra obtained are superimposed and evaluated in a three dimensional space without substantial loss in terms of accuracy and precision. The addition of further dimensions to chromatographic separations by hyphenated techniques offers unique opportunities of efficiently supporting and ensuring the quality and safety of pharmaceuticals [40]. Mass spectrometry is widely used for characterization and identification of impurities and degradation products in pharmaceuticals [41]. Liquid chromatography combined

with mass spectrometry (LC-MS) is considered as one of the most important techniques of the last decade of 20th century [42]. It became the method-of-choice for analytical support in many stages of quality control and assurance with in the pharmaceutical industry [43,44].

3. Recent trends in development of HPLC methods for monitoring of process impurities in bulk drugs

Currently the vast majority of drug-related impurity determinations are performed by HPLC. It offered the desired sensitivity for trace level determinations with a high degree of automation. A wide variety of stationary phases and operation modes make HPLC applicable to all drug classes. The typical detection limits for drug-related impurities by HPLC are 0.1% or lower and this can be routinely met in the majority of circumstances using conventional UV detectors. During the period of review, HPLC was used extensively in the evaluation and control of purity of chemical components used in the manufacture of bulk drugs [45,46]. A variety of approaches to establish the impurity profiles of synthetic drugs have been outlined [47]. These methods involved the prediction of likely impurities with in the synthetic process, their isolation and identification by suitable analytical techniques. HPLC studies on efavirenz and ramipril provide excellent examples of these approaches [48,49]. However, these studies are valid only for materials synthesized by specific routes. Any changes in the synthetic route may lead to a different profile of impurities and must be investigated accordingly. Methods based on some form of HPLC separation still out number of all other assays as they have for over two decades. The bulk of these are relatively simple, isocratic reversed-phase procedures that employ UV detection. However, in some instances, more novel approaches are used that are often based on separation mode or the use of more selective detective systems typically diode array and fluorescence detectors or mass spectrometers [50]. Of these, the application of coupled LC-MS continues to increase in popularity, as electorospray ionization and atmospheric pressure ionization interfaces became more reliable at higher throughput volumes [51,52]. Several approaches using HPLC for determination of impurities in bulk drug substances and their formulations have been reported and the details of these methods are recorded in Table 2 and Table 3 [53–216]. Fig. 7 shows the classification of various drugs analyzed by HPLC for determination of impurities in bulk and formulated materials. In this review we discuss the present state-of the-art of HPLC for some important analgesic, antibiotic, anti-viral, anti-hypertensive, anti-depressant, gastro-intestinal and anti-neoplastic agents in brief.

3.1. Analgesic, antipyretic and anti-inflammatory drugs

As a broad class of therapeutics, analgesic, antipyretic and anti-inflammatory drugs are used not only for headaches but also for arthritis and rheumatic fevers. Because of their widespread use, the quality and safety of these drugs is of considerable importance in the pharmaceutical industry. During the period of review, a variety of analytical procedures have been developed for the more common compounds such as acetaminophen [53] and beclomethasone dipropionate [54] using HPLC.

Pholcodine [5R, 6S)-4,5-epoxy-9α-methyl-3-(morpholinoethoxy)-morphin-7-en-6-ol] is a semi-synthetic opium alkaloid, widely used as an anti-tussive agent. It is synthesized by treating an aqueous solution of morphine with an equivalent amount of sodium hydroxide followed by the addition of chloroethyl morpholine hydrochloride. The possible impurities in pholcodine are shown in Fig. 8. Denk et al. have developed a quantitative HPLC method for separation and determination of structurally related manufacturing impurities including morphine, on a 5 μm Luna phenyl–hexyl column (15 cm × 4.6 mm i.d.) with a mobile phase containing a mixture of acetonitrile:THF:water (10:7:83 v/v) in 20 mM-phosphate buffer at pH 8 [55]. A representative HPLC chromatogram of pholcodine and its impurities is shown in Fig. 9. The method was fully validated and compared with MEKC with regard to selectivity, linearity, precision, robustness, limits of detection and

Table 2
HPLC methods for determination of impurities in bulk drugs appeared in the analytical abstracts during 1995–2001

S. No	Drug	Column	Mobile phase	Detector	Impurities	Refs.
1	Acyclovir	Spherisorb ODS (25 cm × 4.6 mm i.d.)	Methanol/5 mM monopotassium dihydrogen phosphate pH-3 (1:19 v/v)	UV 254 nm	Guanine	[77]
2	Albendazole	Inertsil C_{18} 5 μm (25 cm × 4.6 mm i.d.)	Methanol–water–perchloric acid (45:55:0.3 v/v/v)	UV 254 nm	Albendazole sulfoxide and albendazole sulfone	[80]
3	Amidaraone	Hypersil Nitrile 3 μm (15 cm × 4.6 mm i.d.)	Acetonitrile/0. 1 M ammonium acetate buffer (1:1)	UV 240 nm	Impurities adj. to pH 6 with 0.1 M acetic acid	[174]
4	Amino steroid	Inertsil C_{18} 5 μm (25 cm × 4.6 mm i.d.)	Acetonitrile/THF/ammonium formate/formic acid-buffer pH 3 (81:9:10:0-A)	UV 254 nm	Degradation products in H_2O and (5:4:0.6:10:84-B)	[151]
5	Amoxycillin	C_{18}, C_8 poly (styrene/divinylbenzene)	Acetonitrile/0.05 M phosphate buffer of pH 5 (1:24 v/v)	UV 254 nm	Related substances	[163]
6	Artemisinin	Phenomenex C_{18} (25 cm × 5 mm i.d.)	Aqueous 80% methanol	Evaporative light scattering detection at 32 °C	Related analogues IBO–SIL	[128]
7	Azathioprine	Shim-pack CLC-ODS 5 μm (15 cm × 4.6 mm i.d.)	Acetonitrile/water (30:70 v/v)	UV 210 nm	6-Mercaptopurine and 5-chloro-1-methyl-4-nitroimidazole	[93]
8	Becliconazole	Chiral pak A D (25 cm × 4.6 mm i.d.)	Diethylamine/ethanol/hexane (1:50:950 v/v/v)	UV 220 nm and 254 nm	Potential impurities	[147]
9	Beclomethasone dipropionate	Lichrosorb RP-18 (25 cm × 4.6 mm i.d.)	50–60% acetonitrile in 5 min to aqueous 80% acetonitrile	UV 239 nm	Degradation products	[54]
10	Benazepril HCl	Hichrom RPB C_{18} 5 μm (25 cm × 4.6 mm i.d.)	Aqueous 0.025 M sodium dihydrogen-phosphate buffer pH 2.8/Acetonitrile/methanol (5:3:2 v/v/v) gradient elution	UV 210 nm	Benazepril isomer, prefinal isomer, 1-ter-butylox carbonyl methyl-3-(S)-2,3,4,5-tetrahydro-1H-[1]-benzaze-Pin-2-one, Ethyl (+) R-2-(nitrobenzene sulfonyoxy-4-phenylbutyrate, and benzeprilat	[83]
11	Benzathaine benzyl penicillin	ODS, 5 μm (25 cm × 4.6 mm i.d.)	Phosphate buffer pH 3.5/methanol/water (2:7:11 v/v/v)	UV 220 nm	Impurities	[119]
		Hypersil BDS 5 μm (15 cm × 4.6 mm i.d.)	Phosphate buffer pH 3.5/methanol/H_2O (2:7:11)-A (1:6:3 v/v)-B	UV 205 nm	Impurities	[125]
12	Bisnafide	Zorbax RX–C_8	0.1 M TFA/methanol (97:3)-A and 0.11 M TFA/methanol (4:1 v/v)-B	UV 215 nm	Dimethyl sulfoxide	[144]
13	Butalbital	μ-Bondapak C_{18} (30 cm × 7.8 mm i.d.)	Water:acetonitrile (3:2 v/v)	UV	Process impurities	[122]
14	Bromazepam	ODS C_8 5 μm (25 cm × 4.6 mm i.d.)	Methanol/acetonitrile/5 mM potassium dihydrogen phosphate/0.1 M ammonium acetate buffer (26.5:21.5:52 v/v/v)	UV 239 nm	Degradation products	[136]
15	Budesonide	Hypersil C_{18} 5 μm (25 cm × 4.6 mm i.d.)	Ethanol–acetonitrile–phosphate buffer (pH 3.4; 25.6 mM) (2:30:68 v/v/v)	UV 240 nm	Impurities/degradation products	[186]

Table 2 (*Continued*)

S. No	Drug	Column	Mobile phase	Detector	Impurities	Refs.
16	Calicoprtriol	RP-18	Methanol/acetonitrile/water (67:23:10 v/v/v)	UV	Cholecalciferol and calcitriol	[120]
17	Cefdinir	TSK gel ODS-80 TM 5 μm (7.5 cm × 4.6 mm i.d.)	33 mM citrate–phosphate buffer pH 2/methanol/dioxane (36:4:1 v/v/v)	UV 254 nm	Related substances	[74]
18	Cephradine	Bio-Gel PRP 8 μm (25 cm × 2.1 cm i.d.) Preparative column	0.01 M acetic acid/acetonitrile (47:3 v/v)	UV 254 nm	4′,5′-dihydro cephradine	[173]
19	Ceftriaxone	Kromasil C_{18} (20 cm × 4.6 mm i.d.) 5 μm	Acetonitrile/tetrabutyl ammonium bromide/phosphate buffer pH 7/H_2O (400:4:55:795 v/v/v/v/v)	UV 270 nm	7-Aminocephalosporanic acid, 7-amino-3[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-1,2,4-triazin-3-yl) thiomethyl-3-cephem-4-carboxylicacid	[71]
20	Celicoxib	Novapak C_{18} 4 μm (30 cm × 3.9 mm i.d.)	0.01 M potassium dihydrogen phosphate (pH 4.8)-acetonitrile (45:55 v/v)	UV 252 nm	5-Methyl-2-nitro phenol, 4-hydrazino benzene sulfonamide 1-(4-methyl phenyl)-4,4,4-trifluro butan-1,3-dione	[59]
21	Chlorhexidine Gluconate	Nucleosil C_{18}, 3 μm (15 cm × 4.6 mm i.d.)	0.1 M ammonium acetate/H_2O adj. to pH 5 with acetic acid-A and acetonitrile-B	UV 320 nm	Degradation products	[156]
22	5-Chlorooxindole	Novapak 4 μm (15 cm × 3.9 mm i.d.)	Hexane/IPA/THF/15-crown 5 (2000:12:12:1 v/v/v/v)	UV 254 nm	6-chlorooxindole, oxindole, 4-chlorooxindole, 5,7-dichlorooxindole and 7-chlorooxindole	[161]
23	Chlorprothixene hydrochloride	Hypersil C_{18}, BDS 3 μm (10 cm × 4 mm i.d.)	H_2O/acetonitrile/methanol (11:8:1) containing 6 g/l KH_2PO_4, 2.9 g/l sodium lauryl sulfate and 9 g/*n*-butyl ammonium bromide	PDA 254 nm	E and Z isomers, 2-chlorthioxanthone, 2-3-2-chloro-9H-thioxanthane-9-ylidene-*N*-methylpropyl amine, 3-(2-chloro-9H-thioxanthan-9-ol-*NN*-dimethyl propyl amine and 2-3-(4-chloro-9H-thioanthen-9-ylidene-*NN*-di-methyl propyl amine	[158]
24	Chlorthiazide	Hibar Lichrosorb RP-18 5 μm (25 cm × 4.6 mm i.d.)	Acetonitrile/water/acetic acid (5:44:1 v/v/v)	UV 280 nm	Decomposition products	[169]
25	Cimitidine	Nucleosil C_{18}, 10 μm (25 cm × 4 mm i.d.)	270 ml methanol, 0.3 ml 85% H_3PO_4 and 0.94 g. sodium 1-hexane-sulfonate and diluted to 1 l with water	PDA 220 nm	Impurities	[87]
26	Ciprofloxacin	Flexit ODS (30 cm × 3.5 mm i.d.)	Methanol–water–acetic acid (84:15.9:0.1 v/v/v)	UV 254 nm	Chlorofluoroaniline, dichloroacetophenone, chloropropylacrylate and Q-acid	[75]

Table 2 (*Continued*)

S. No	Drug	Column	Mobile phase	Detector	Impurities	Refs.
		Novapak C_{18}	20 mM H_3PO_4 pH 2.3/acetonitrile (17:3 v/v/) containing 2.5 mM sodium heptane-1-sulfonate OR 20 mM H_3PO_4 pH 2.3/THF (47:3 v/v) containing 0.25 mM sodium heptane-1-sulfonate	UV 278 nm	Degradation products	[181]
27	Clindamycin	Hypersil ODS 5 μm (25 cm × 4.6 mm i.d.)	acetonitrile/phosphate buffer (1. 35% phosphoric acid adj. to pH 6 with ammonia)/water (7:8:5 v/v/v)	UV 210 nm	Lincomycin, Lincomycin B 7-epiclindamycin, clindamycin B, and 7-epilincomycin	[68]
28	Clodronic acid	Ion pac AS 5	NaOH (20–100 mM) and a self regenerating ASRS-1 suppressor	Conductivity	Impurities	[109]
29	Clofazimine	Beckman Ultra-sphere 5 μm (25 cm × 4.6 mm i.d.)	Sodium lauryl sulfate (2.25 g) tetrabutyl ammonium hydrogen sulfate (0.85 g), disodium hydrogen phosphate (0.885 g) in 490 ml water pH 3 with H_3PO_4 made up to 500 ml	UV 280 nm	Iminophenazine and iminophenazine base	[118]
30	Clonazepam	Lichrospher RP-8 (12.5 cm × 4 mm i.d.) 5 μm	0.05 M K_2HPO_4, pH 2.7 with H_3PO_4/acetonitrile (3:2 v/v)	UV 215 nm	3-amino-4-(2-chloro-phenyl)-6-nitrocarbostyril and 2-amino-2′-chloro-5-nitrobenzophenone	[140]
31	Cocarboxylase	Lichrospher 100 RP-18 5 μm (12.5 cm × 4 mm i.d.)	10 mM KH_2PO_4 containing 6.2 mM tetrabutyl ammonium bromide at pH 6.8	UV 233 nm	Phosphothiamin	[149]
32	Cromlyn sodium	C_8 Novapak (15 cm × 3.9 mm i.d.)	Methanol/1 M tetra butyl ammonium hydrogen phosphate buffer (9:11 v/v)	UV 326 nm	Chromlyn diethyl ether and hydroxy phenoxy-2-propanol	[104]
33	Dantrolene sodium	Zorbax C_{18} column 5 μm (25 cm × 4 mm i.d.)	Water–methanol–acetonitrile (2:1:2 v/v/v)	UV 315 nm	Acetonesemicarbazone, *p*-nitroaniline and 3-nitro phenyl furfural	[187]
34	Dexfenfluramine	Nucleosil–NH_2 (20 cm × 4 mm i.d.) and Chiracel OF (25 cm × 4.6 mm i.d.)	–	UV 265 nm	Fenfluramine and isomeric impurities	[159]
35	Diamine	RP-18/cation column 5 μm (15 cm × 4.6 mm i.d.) 3 M Z-18 column (15 cm × 4.6 mm i.d.) Zorbax RX C_8 5 μm (25 cm × 4.6 mm i.d.)	50–100% methanol versus 10 mM sodium phosphate pH 3.5 50–100% methanol versus sodium phosphate plus 100 mM ammonium chloride pH 5.8	Diode array	Process impurities	[177]
36	Diamorphine	Hypersil BDS C_{18} 5 μm (25 cm × 4.6 mm i.d.)	0.05 M KH_2PO_4 of pH 3/acetonitrile (4:1 v/v)	UV	6-Acetyl morphine and morphine	[170]
37	Diatrizoate sodium	PRP— × 100	0.1 M KCl/0.05 M di basic/potassium phosphate in H_2O/acetonitrile (9:1 v/v)	UV 232 nm	2,4-and 2,6-di iodo 3,5-diacetamido-benzoic acid and 5-acetomido-3-amino-2,4,6-tri-iodo benzoic acid	[107]

Table 2 (*Continued*)

S. No	Drug	Column	Mobile phase	Detector	Impurities	Refs.
38	Diaziridinyl Benzoquinone	Inertsil C_8 5 μm (25 cm × 4.6 mm i.d.)	Methanol and NH_4OAc (pH 4.0; 20 mM) (30:70 v/v)	UV 330 nm	Degradation products	[185]
39	1,3-Dichloro 6,7,8,9,10,12-hexa hydrasepino [2,1-b] quinazolin mono hydrochloride	Water symmetry base deactivated C_{18}, 5 μm (25 cm × 4.6 mm i.d.)	Methanol/0.03 M S.D.S. of pH 4 (17:8 v/v)	UV 228 nm	Potential impurities	[148]
40	Dicloxacillin sodium	ODS (25 cm × 4.6 mm i.d.)	0.27% phosphate buffer pH 5/acetonitrile (3:1 v/v)	UV 225 nm	Impurities	[69]
41	Efavirenz	Zorbax SB-CN column (15 cm × 4.6 mm i.d.)	90% water, with 0.05% trifluroacetic acid, 10% methanol (A) 90% methanol, 10% water, with 0.05% trifluroacetic acid (B) gradient elution	UV 250 nm	Methyl DMP 266 SV 997, SD 573-methyl carbamate SV 993, SD 572 SG 275 and SW 965	[48]
42	Enrofloxacin	Shodex Micro C_{18}, 5 μm (15 cm × 3.9 mm i.d.)	0.025 M phosphoric acid buffer/acetonitrile (17:3 v/v) pH 2.8–3 with triethyl amine	UV 285 nm	Process impurities	[166]
43	Erithromycin	Prodigy ODS 2	Phosphate buffer, tetra butyl ammonium hydrogen sulfate and acetonitrile	–	Related impurities	[117]
44	Famotidine	Spherisorb C_8 10 μm (30 cm × 3.5 mm i.d.)	acetonitrile-0.01 M aqueous potassium dihydrogen phosphate (25:75 v/v) pH 3.15	UV 254 nm	Thiourea, chloro-*N*(aminosulfonyl) propanimidoamine, diamino methylene amino (chloromethyl) thiozole and diaminomethyleneamino (1-amino-1′-iminomethelene)thio-methyl thiazole	[88]
45	Fenbendazole	Inertsil C_{18} 5 μm (25 cm × 4.6 mm i.d.)	Orthophosphoric acid (0.1% v/v in H_2O and acetonitrile (40:60 v/v) pH adjusted to 4.0 with 1.0 M KOH	UV 290 nm	Methyl benzimidazolecarbamate, 5-thiophenyl-2-methyl benzimidazole, 5-thiophenyl benzimidazole	[80]
46	Fenofibrate	Symmetry ODS (10 cm × 4.6 mm) 3.5 μm	Aqueous 70% acetonitrile containing 0.1% TFA	UV 280 nm	Impurities	[123]
47	Finasteride	Supelcosil LC-18 DB column (25 cm × 4.6 mm i.d.)	THF/methanol/TFA/water (3:5:0.25:65 v/v/v/v)	UV 210 nm	Related compounds	[90]
48	Flucloxacillin	ODS 5 μm (25 cm × 4 mm i.d.)	Phosphate buffer of pH 5/acetonitrile (3:1 v/v)	UV 225 nm	Related substances	[141]
49	Fluoxetine HCl	Spherisorb Nitrile 3 μm (15 cm × 4.6 mm i.d.)	THF/acetonitrile/0.25% TFA (1:3:16 v/v/v)	UV 214 nm	Related compounds	[101]
50	Haloperidol	ODS-I 1.5 μm non porus silica (3.3 cm × 4.6 mm i.d.)	Buffer (3.42 g KH_2PO_4 in 500 ml H_2O, 1 ml triethylamine pH 2.5/acetonitrile (77:23 v/v)	UV 220 nm	4, 4′-bis[4-(*p*-Chlorophenyl)-hydroxy piperidine] butyro-phenone	[102]

Table 2 (*Continued*)

S. No	Drug	Column	Mobile phase	Detector	Impurities	Refs.
51	Hydroxyzine HCl	Lichrosorb RP-18 Hypersil ODS Nucleosil 100-5 C_{18} all (12 cm × 4 mm i.d.)	0.35% sodium methane sulfonate in 9.8% aqueous triethylamine at pH 2. 85/acetonitrile	UV 230 nm	1-[(4-chloro-phenyl) phenyl methyl) piperazine and deschlorohydorxyzine	[164]
52	Ipriflavone	C_{18} 3 μm (30 cm × 4.6 mm i.d.)	0.01 M triethylamine adj. to pH 2.5 with H_3PO_4/acetonitrile (1:1 v/v)	UV 225 nm	Benzyl 2-hydroxy-4-isopropoxy phenyl ketone, 7-hydroxy-isoflavone, benzyl 2,4-di-isopropoxy phenly ketone and 7-ethoxy isoflavone	[155]
53	Irinotecan	Altima C_{18} 5 μm (25 cm × 4.6 mm i.d.)	Acetonitrile/0.1 M KH_2PO_4 buffer of pH cont. 25.7 mM triethylamine (258:742 v/v)	UV 254 nm	Camptothecin (CPT), 7-ethyl-CPT, 7-ethyl-1-o-CPT, CPT-11-carboxylic acid and SN-38-carboxylic acid	[116]
54	Isepamicin	Two Waters X-Terra RP 18 3.5 μm (5 cm × 4.6 mm i.d.) (15 cm × 4.6 mm i.d.)	Water/ammonium hydroxide/acetic acid (96:3.6:0.4 v/v/v)	Evaporative light scattering detector	Potential impurities and degradation products	[76]
55	Isometamedium chloride	Lichrospher 60 RP select B (12.5 cm × 4 mm i.d.)	Acetonitrile/20 mM KH_2PO_4 buffer pH 3 (1:3 v/v)	UV 320 nm	Process and degradation impurities	[121]
56	Isosorbide 5-mononitrate	Lichrosorb RP 18 5 μm (25 cm × 4 mm i.d.)	Methanol/water (3:7 v/v)	UV 220 nm	Inorganic nitrate	[175]
57	Leuprolide	Nucleosil C_{18}	Acetonitrile/water (85:15 v/v)	UV	Fluoride	[89]
58	Lincomycin	Supelcosil LC-ABZ C_{12}-C_{18}, 5 μm (25 cm × 4.6 mm i.d.)	2.25% acetonitrile, 2.72% KH_2PO_4 (pH 5) with 3. 48% KH_2PO_4 and 0.067% methane sulfonic acid in H_2O	UV 210 nm	Lincomycin B and 7-epilincomycin	[68]
59	Lorazepam	BDS C_8 5 μm (25 cm × 4.6 mm i.d.)	Methanol/acetonitrile/buffer (0.005 M KH_2PO_4/0.1 M adj. pH 6 with acetic acid (7:4:9 v/v/v)	UV 230 nm	6-Chloro-4-(2-chloro-Phenyl)-2-quinazoline carboxaldehyde	[134]
60	Lovastatin	Nucleosil ODS 7 μm (25 cm × 4.6 mm i.d.)	Borax buffer pH 4/methanol (3:17 v/v)	UV 230 nm	Impurities	[108]
61	Mangafodipir	PRP-1 5 μm (15 cm × 4.6 mm i.d.)	Acetonitrile-borate/terabutyl ammonium hydrogensulfate (pH 9.9; 13 mM borate, 36 Mm TBA) (25:75, v/v)	UV 310 nm	Manganese(III) dipyridoxyl diphosphate sodium salt, manganese dipyridoxylmonophosphate sodium salt, manganese dipyridoxyl diphosphate monooveralkyated sodium salt and manganese (5-methyl) dipyridoxyl monophosphate sodium salt	[112]
62	Mebendazole	Inertsil C_{18} 5 μm (25 cm × 4.6 mm i.d.)	Orthophosphoric acid (0.1% v/v in H_2O and acetonitrile (70:30 v/v) pH adjusted to 6.0 with 1.0 M KOH	UV 290 nm	2-amino-5-benzoyl-benzimidazole, methyl benzimidazole-carbonate, 2-hydroxy-5-benzoyl-benzimidazole, 2-methyl-5-benzoyl-benzimidazole	[80]
63	Mefenamic acid	Nucleosil C_{18} 5 μm (25 cm × 4.6 mm i.d.)	H_2O—pH 3 with H_3PO_4	UV 210 nm	Related substances	[124]

Table 2 (*Continued*)

S. No	Drug	Column	Mobile phase	Detector	Impurities	Refs.
64	Melphalan	BDS Hypersil C_{18} 5 μm (15 cm × 4.6 mm i.d.)	Buffer (acetic acid/triethylamine/ammonium acetate/H_2O; 5:1:5:44—A/acetonitrile/H_2O (1:120:80; B)	UV 260 nm	Related impurities	[92]
65	β-methyl digyoxin	Sphresorb C_{18} 10 μm (15 cm × 4.6 mm i.d.)	Acetonitrile/water (17:33 v/v)	UV 218 nm	Impurities	[85]
66	*N*-methyl-D-aspartate	Zorbax SB-phenyl (25 cm × 4.6 mm i.d.)	Aqueous 1% acetonitrile adj. pH 2.4 with TFA-A; aqueous 60% acetonitrile adj. pH 2.4 with TFA-B	Evaporative light scattering and antagonist UV 205 nm	Bi phosphonic acid and 6-methyl derivate	[152]
67	Mexidole and Emoxipine	ODS-100 5 μm (25 cm × 4 mm i.d.)	0.01 M NaH_2PO_4 (9:1 v/v) pH 3 with H_3PO_4/acetonitrile containing 50 mg 1-sodium octyl sulfonate	UV 296 nm	Impurities	[145]
68	Midecamycin acetate	YWG-C_{18} 10 μm (20 cm × 4 mm i.d.)	Phosphate buffer of pH 7.1/methanol (27:73 v/v)	UV 232 nm	Impurities	[154]
69	Naproxen	Shim-pack CLC-ODS (25 cm × 4.6 mm i.d.)	Aqueous 80% methanol containing 50 mM lactic acid/HCl O_4 of pH 2.5	UV 271 nm	5-Bromo naproxen	[135]
70	Naproxen & Pseudoephedrine HCl	Sperisorb cyano 5 μm (25 cm × 4.6 mm i.d.)	Triethylamine/methanol/water (1:15:15:170 v/v/v/v)	UV 260 nm and 235 nm	Impurities	[67]
71	Nefazodone HCl	Inertsil ODS-3V (25 cm × 4.6 mm i.d.)	0.05 M KH_2PO_4 (pH 3.0)/acetonitrile/methanol (50:40:10 v/v/v)	UV 220 nm	5-Ethyl-4-(2-phenoxyethyl)-2H-1,2,4-triazol-3-(4H)one, 1-(3-chlorophenyl)-4-(3-chloropropyl) piperazine HCl and 1,1′-trimethylene-bis[4-(3-chlorophenyl piperazine]hydrochloride	[97]
72	Nevirapine	Supelcosil	Acetonitrile/25 mM $NH_4H_2PO_4$, pH 5 (1:4 v/v)	UV 220 nm	Related impurities	[78]
73	Nicotine	Supelcosil LC-ABZ, 5 μm (25 cm × 4.6 mm i.d.)	Potassium dihydrogen phosphate buffer neutralized to pH 7.1 with 1 M KOH containing 1 mM triethylamine/acetonitrile (17:3 v/v)	UV 254 nm	Cotinine, nicotine 1′ oxide and nicotinic acid	[138]
74	Nimodipine	C_{18} Nucleosil 10 μm (25 cm × 4.6 mm i.d.)	Aqueous 75% methanol	UV 254 nm	bis-(1-Methyl ethyl) and bis (2-methoxy ethyl)2,6-dimethyl-4-(3-nitro phenyl) 1,4-dihydro-pyridine-3,5-dicarboxylate	[114]
75	Nitrendipine	Lichrosorb RP-18 5 μm (25 cm × 4 mm i.d.)	Methanol–water (70:30 v/v) pH 3 adjusted to 3 with H_3PO_4	UV 238 nm	Methyl-3-amino crotonate, 2-ethyl-2-(nitro-benzylidene) acetoacetate, 1,4-dihydro-2,6-dimethyl-4-(3-nitro-phenyl)-3,5-pyridinedicarboxylic acid, diethyl ester and 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)3,5-pyridine dicarboxylic acid dimethyl ester	[81]
76	Oestradiol	Lichrosorb RP-18 (25 cm × 4.6 mm i.d.)	Acetonitrile/water (7:3 v/v)	UV 210 nm	Impurities	[22]

Table 2 (*Continued*)

S. No	Drug	Column	Mobile phase	Detector	Impurities	Refs.
77	Olpadronate	Waters IC Pak HR 6 μm	Dilute nitric acid (18 mΩ water to pH 2.9 with 10% HNO_3)	UV 235 nm with reversed polarity	Phosphite, phosphate, chloride and methane sulfonic acid	[111]
78	Orbofiban	YMC basic 5 μm (15 cm × 4.6 mm i.d.)	Methanol and acetonitrile 10 mM phosphate buffer pH 7.0 Gradient elution	UV 280 nm	Related compounds	[6]
79	Ornidazole	Supelcosil LC-18-DB (25 cm × 4.6 mm i.d.)	Water–acetonitrile (86:14 v/v)	UV 310 nm	Degradation products	[188]
80	Ostrogens	Hypersil 5 ODS Prodigy 5 ODS 2	Triethylamine phosphate buffer	Irradiated at 254 nm for fluorimetric detection at 410 nm	Impurities	[137]
81	Oxaprozin	Inertsil-ODS 3V 5 μm (15 cm x4.6 mm i.d.)	Phosphate buffer-acetonitrile gradient elution	UV 254 nm	Process impurities	[61]
82	3-Oxo steroids prednisolone	Li chrospher 100 RP 18, 10 μm (12.5 cm × 4 mm i.d.)	A. 0.1 M aq. ammonium acetate/water/methanol (10:80:10 v/v/v) B. aq. Ammonium acetate/ methanol (10:90 v/v) gradient	UV 240 nm	Impurities	[113]
83	Oxybutynin chloride	Supelcosil LC-8-DB (25 cm × 4.6 mm i.d.) 5 μm	Acetonitrile/triethanolamine phosphate buffer of pH 3.5 (1:1 v/v)	UV 200 nm	Chloride	[133]
84	Paclitaxel	Meta Chem Taxsil 5 μm (15 cm × 2 mm i.d.)	Acetonitrile-A and water/methanol (9:1 v/v-B)	UV 220 nm	Degradation products	[146]
85	Permethrin	C-18 Nova-Pak 4 μm (15 × 3. 9 mm i.d)	Methanol–water (78:22 v/v)	UV 272 nm	Cis and trans-isomers, impurities and degradation products	[110]
86	Perphenazine trifluoperazine triflupromazine	Novapak phenyl 4 μm (15 cm × 3.9 mm i.d.)	Methanol/5 mM sodium acetate pH 6.5 (81:19 v/v)	UV 254 nm	Degradation products	[157]
87	Pholcodine	Luna phenyl-hexyl column 5 μm (15 cm × 4.6 mm i.d.)	Acetonitrile 10% (v/v), THF 7% (v/v) in 20 mM phosphate buffer pH 8	UV 238 nm and 314 nm	Potential manufacturing impurities including morphine	[55]
88	Phystigmine salicylate	Bondapak C_{18} 10 μm (25 cm × 5 mm i.d.)	Acetonitrile/0.1 M ammonium acetate buffer pH 6 (1:1 v/v)	UV 305 nm	Degradation products	[126]
89	Pirmenol HCl	YMC-PMC AM-312 ODS (15 cm × 6 mm i.d.)	50 mM Ammonium phosphate buffer of pH 7.2/acetonitrile/acetic acid	UV	3-(Cis-2,6-diemethyl piperidinlyl) propyl 2-(2-pyridyl) phenyl ketone, 2-(2-pyridyl) benzoic acid and methyl 2-(2-pyridyl) benzoate	[176]
90	Procaine, adiphenine, drofenine, nafranyl, tetracaine and meclofenoxate	Sperisorb ODS 2 (12.5 or 25 cm × 5 mm i.d.)	Acetonitrile or methanol and 0.02 M sodium acetate/acetic acid buffer pH 4.5	UV variable wavelength	Degradation products	[131]
91	Procainebenzyl penicillin	ODS (25 cm × 4.6 mm i.d.)	750 volumes of a solution containing 7 g. KH_2PO_4 and 3.25 g tetrabutylammoniumhydroxide pH 7.2 and 250 ml acetonitrile	UV 225 nm	Impurities	[132]

Table 2 (*Continued*)

S. No	Drug	Column	Mobile phase	Detector	Impurities	Refs.
92	Ranitidine	Ultrasphere ODS C_{18} (25 cm × 4.6 mm i.d.)	Acetonitrile/10 mM potassium phosphate buffer of pH 5 (2:23 v/v)	UV 262 nm	Ranitidine-*S*-oxide and degradation products	[179]
		YMC-pack ODS-AM 5 μm (15 cm × 4.6 mm i.d.)	10 mM SDS, 50 mM H_3PO_4 pH 6.8 with TEA and 43% methanol	UV 228 nm	[5-[[(2-Aminoethyl)-thio]methyl]-N, N-dimethyl-2-furanmethanamine, (*N*,*N*′-bis[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl] 2-nitro-1,1′-ethenediamine and ranitidine-*S*-oxide	[86]
93	Ribostamycin	Perkin–Elmer 3 × 3 CR_{18} 3 μm (3.3 cm × 4.6 mm i.d.)	0.01 M Na_3PO_4 buffer pH 4.5/methanol/acetonitrile (25:1:24 v/v/v)	Diode array 230 nm	Neaamine	[153]
94	Rocicoxib	Symmetry C_{18} 5 μm (25 cm x4.6 mm i.d.)	Acetonitrile–water (50:50 v/v)	UV 225 nm	Process impurities	[60]
95	Rooperol tetra acetate	C_{18} 10 μm	Acetonitrile/water (7:3 v/v)	UV 260 nm	Degradation products	[129]
96	Ropinirole	Nucleosil 100-5 C_{18} micro column	8.7 mM MES pH 6 with Na OH/Acetonitrile (9:11 v/v)	UV 254 nm	Synthetic impurities	[105]
97	Roxithromycin	ODS (25 cm × 4.6 mm i.d.)	Acetonitrile/water/ammonium dihydrogen phosphate (26%) pH 4.3	–	Related substances	[115]
98	Rutin	Lichrosorb RP C_8 10 μm (25 cm × 4.6 mm i.d.)	0.1 M NaH_2PO_4 buffer of pH 3 (buffer A) THF (19:1) as solvent A and buffer A/THF (3:2) as solvent B	UV 280 nm	Quercitrin, hyperoside and isoquercitrin	[160]
99	Salbutamol	Waters μ-Bondapak C_{18} (30 cm × 3.9 mm i.d.)	Methanolic 80% 5 mM sodium hexane sulfonate in 1% glacial acetic acid	UV 280 nm or Amperometric, glassy carbon electrode at 1 V Ag/AgCl	Degradation products	[150]
100	Salycilic acid	Supelcosil LC-18 LC-18—DB	Methanol/water/acetonitrile (40:60:1 v/v/v)	UV 270 nm	4-Hydroxy benzoic acid, phenol and 4-hydroxy iso pthalic acid	[171]
101	Sildenafil citrate	μ-Bondapak C_{18} 4 μm (30 cm × 3.9 mm i.d.)	0.02 M ammonium acetate (pH 7.0)—acetonitrile (1:1 v/v)	UV 240 nm	1-Methyl-4-nitro-3-*n*-propyl-5-pyrazolecarboxamide, 4-amino-1-methyl-3-*n*-propyl-5-pyrazole-carboxamide, 4-(2-ethoxybenzoyl-amino)-1-methyl-3-*n*-propyl-5-pyrazole-carboxamide, 5-(2-ethoxy-phenyl)-1-methyl-3-*n*-propyl-pyrazole[4,3-d]pyrimidine-7-1	[184]
		LiChrospher C_{18} (25 cm × 4.6 mm i.d.) 5 μm	70 mM potassium phosphate monobasic buffer of pH 3 containing 100 mM triethylamine/acetonitrile (7:3 v/v)	UV 225 nm	Oxidative induced degradation products	[139]
102	Sodium cephazolin	Spherisorb ODS 5 μm (15 cm × 4.6 mm i.d.)	Methanol/0.02 M citrate buffer of pH 3.2 (11:39 v/v)	UV 254 nm	7-Amino cephalosporanic acid and 7-amino cephalosporanic acid-MT	[178]

Table 2 (*Continued*)

S. No	Drug	Column	Mobile phase	Detector	Impurities	Refs.
103	Tetracycline	PLRP-5 C_{18} 8 μm (25 cm × 4.6 mm i.d.)	2-Methyl-2-propanol/0.2 M sodium phosphate buffer pH 9/0.02 M tetrabutylammonium hydrogen sulfate of pH 9/0.01 M EDTA of pH 9 (7:5:10:15:10 v/v/v) diluted to 100 parts by volume with water	UV 254 nm	Epitetracyclin, epianhydrotetracycline, anhydrotetracycline and 2-acetyl-2-de carboxamido-tetracycline	[172]
		Chromspher C_8	Acetonitrile/0.01 M oxalic acid of pH 2	Fluorescence	Epitetracycline, 4-epianhydro tetracycline and anhydrotetra tetracycline	[127]
104	Terazosin	Spherisorb C_{18} 10 μm (25 cm × 5 mm i.d.)	Acetonitrile/THF/ 10 mM KH_2PO_4 (3:1:16 v/v/v)	UV 222 nm	Impurities	[130]
105	Tiaprofenic acid	Chiralcel OD (25 cm × 4.6 mm i.d.) Chiral pak AD	0.1% TFA/Hexane/propan-2-ol (197:3) or (47:3)	UV 296 nm	2-Acetyl-5-benzoyl thiophene, 5-benzoyl-2-ethyl thiophene and (RS)-5-benzoyl-2-methyl-3-thiophene acetic acid	[183]
106	Ticarcillin sodium	ODS 5 μm (25 cm × 4 mm i.d.)	0.13% ammonium phosphate of pH 7/ methanol (4:1 v/v)	UV 220 nm	Related substances	[142]
		Hypersil ODS 5 μm (25 cm × 4 mm i.d.)	Ammonium phosphate buffer of pH 6/ methanol	UV 220 nm	Impurities	[162]
107	Tofisopam	Lichrosorb 10 RP 18 (25 cm × 4.6 mm i.d.)	Water/acetonitrile/methanol (46:31:26 v/v/v) pH 5 containing M sodium hepatane sulfonate	PDA	Impurities	[168]
108	Tolnaftate	Hypersil Phenyl 5 μm (25 cm × 4.6 mm i.d.)	Water/methanol (2:3 v/v)	UV 250 nm	*N*-methyl-*m*-toluidine and β-naphthol-1-chlorothiocarbamate	[79]
109	Tolrestat	ODS 5 μm (15 cm × 3.9 mm i.d.)	$NH_4H_2PO_4$ buffer pH 3.5 adj. with 0.05 M H_3PO_4/acetonitrile/THF/Tetra butyl ammonium hydroxide (40% in water) (615:205:185:3)	UV	Impurities	[180]
110	Topiramate	Dionex, Ion pac AS 5 A 5 μm (15 cm × 4.6 mm i.d.)	NaOH gradient (2-25 mM)	Conductivity	Sulfamate and sulfate	[103]
111	Trazodone	Sperisorb ODS 5 μm (15 cm × 4.6 mm i.d.)	THF/methanol/acetonitrile/0.5% trifluoro acetic acid (6:2:6:27 v/v/v/v) and Water/ acetonitrile/diethyl/amine (1200:800:1 or 600:1400:1 v/v)	UV 248 nm, UV 254 nm	Related compounds	[167]
112	Trifluoperazine	Bondapak C_{18}, 5 μm	450 ml acetonitrile/0.1% H_3PO_4 (23:17 v/v)	UV 254 nm	Photodegradation products	[165]
113	Trimethoprim	Nucleosil 120-5 C_{18} AB (25 cm × 4.6 mm i.d.)	Methanol/1.4 g/l aqueous sodium perchlorate (3:7 v/v) pH adj. to 3.1 with H_3PO_4	UV 280 nm	Impurities	[63]

Table 2 (*Continued*)

S. No	Drug	Column	Mobile phase	Detector	Impurities	Refs.
		Ultrasphere ODS 5 μm (25 cm × 4.6 mm i.d.)	0.25% TFA/0.1% formic acid (pH 5.8)-acetonitrile-gradient	UV 272 nm	2,4-Diamino-5-(4-ethoxy-3,5-dimethoxy benzyl) pyrimidine and 2,4-diamino-5-(3-bromo-4,5-dimethoxy benzyl) pyrimidine	[7]
114	L-Tryptophan	Nucleosil 120 3-C_{18} (25 cm × 4 mm i.d.)	0.1% TFA, methanol and acetic acid	Detection at 260 nm by measuring fluorescence at 343 nm, 425 nm, 335 nm	Contaminants	[143]
115	Tylosin A	PLRP-8 8 μm (25 cm × 4.6 mm i.d.)	THF/0.2 M potassium phosphate buffer of pH 9/water (4:1:15)	UV 280 nm	Potential impurities	[182]
116	Ursodeoxycholic acid	Hypersil ODS RP-18, 3 μm (10 cm × 2.1 mm i.d.)	Methanol/acetonitrile/water (30:11:9 v/v)pH 4	Evaporative light scattering detector	Ursocholic acid, cholic acid, chenodeoxy cholic acid, deoxycholic acid, litocholic acid	[106]
117	Verapamil HCl	Supelco pK_b 100 5 μm (25 cm × 4.6 mm i.d.)	0.04 M dibasic potassium phosphate of pH 7.2/acetonitrile	UV 278 nm	Related substances	[82]
118	Warfarin & Acenocoumorol	Select B RP-8, 5 μm (25 cm × 4.6 mm i.d.)	Acetonitrile/water acidified to pH 3 with H_3PO_4—gradient	UV 280 nm	4-Hydroxy coumarin and 4-nitrobenzalacetone	[84]

Table 3
HPLC methods for determination of impurities in formulations appeared in the analytical abstracts during 1995–2001

S. No	Drug	Formulations	Column	Mobile phase	Detector	Impurities	Refs.
1	Acetanophen	Tablets	Symmetry ODS 10 μm (25 cm × 4.6 mm i.d.)	Acetonitrile/water (7:3 v/v)	UV 254 nm	*O*-Acetyl acetaminophen, *O*-ethylacetaminophen, *O*-(2-nitrobenzenesulfonyl)acetaminophen, *O*-benzylacetaminophen and *O*-3,5-dinitro benzyl) acetaminophen	[53]
2	4-acetyl amino phenyl acetic acid	Tablets (Actarit)	Kromasil C_{18} 5 μm (25 cm × 4.6 mm i.d.)	70% methanol and 1% tetrabutyl ammonium bromide in water	UV 245 nm	4-Aminophenylacetic acid, 4-nitrophenylacetonitrile and 4-nitrophenylacetic acid	[62]
3	Acylovir	Injection, tablets	Hypersil HP ODS 5 μm (10 cm × 2.1 mm i.d.)	0.02 M ammonium acetate buffer adjusted to pH 4.5	UV 254 nm	Guanine	[210]
4	Aloe	Pharmaceuticals	Lichrosorb RP 18 5 μm (25 cm × 4 mm i.d.)	5 mM phosphate buffer of pH 3 containing 45% methanol and 1% THF	UV 257 nm	Aloe-emodin	[206]
5	5-amino salicylic acid	Suppositories tablets and capsules	Spherisorb S 5 C_8 (25 cm × 4.6 mm i.d.)	Methanol/phosphate buffer of pH 7.4 (1:4 v/v)	UV 290 nm	Degradation products	[208]
6	Ampicillin Cloxacillin	Capsules suspensions and syrups	ODS Hypersil 3 μm (10 cm × 2 mm i.d.)	Acetonitrile/20 mM phosphate buffer of pH 2 (15:85 v/v) containing 100 mM S.D.S.	UV 230 nm	Precursors and degradation products	[73]
7	Amoxycillin sodium	Injections	Lichrospher RP-18 10 μm (25 cm × 4 mm i.d.)	0.01 M KH_2PO_4 of pH 6 (A)/acetonitrile(1:4 v/v) (B)	UV 215 nm	Amoxicillioic acid, amoxycillinpiperazine-2,5-dione and amoxicillin dimer and tetramer	[201]
8	Aspirin	Aerosol	Econosphere C_8 5 μm (25 cm × 4.6 mm i.d.)	Methanol/THF/1 M H_3PO_4/water (44:5:5:46 v/v/v/v)	UV 275 nm	Salicylic acid, acetyl salicylic acid and salicyl salicylic acid	[57]
9	Atenolol	Tablets	C_{18} Alltima (15 cm × 4.6 mm i.d.)	TEA acetate(pH 4; 0.01 M)/acetonitrile (96:4 v/v)	UV 220 nm, 270 nm, 335 nm	2-(4-Hydroxyphenyl)acetamide	[191]
10	Azithromycin	Capsules	μ-Bondapak C_{18} 10 μm	67 mM KH_2PO_4 buffer pH 4/acetonitrile (7:3 v/v)	UV 210 nm	Degradation products	[194]
11	Bifonazole	Cream	Hypersil ODS 5 μm (20 cm × 4.6 mm i.d.)	Methanol/aqueous 80 mM triethylammoniumphosphate buffer of pH 7/acetonitrile (7:2:1 v/v/v)	UV 254 nm	Degradation products	[216]
12	Carbamazepine	Tablets	Fine Pak C_8 (25 cm × 4.6 mm i.d.)	Aqueous 70% methanol	UV 254 nm	5H-dibenz(b_2f)azepine, acridine, 9 (10H)-acridinone, acridine-9-carboxylic acid and 9-methyl acridine	[204]

Table 3 (*Continued*)

S. No	Drug	Formulations	Column	Mobile phase	Detector	Impurities	Refs.
13	Chlorhexidine	Ophthalmic solution	Hamilton PRP-1 (25 cm × 4.1 mm i.d.) 10 μm	10–50% acetonitrile in 0.02 M ammonium acetate or phosphate buffer pH 5	UV 235 nm	Hydrolysis products	[211]
14	Ciftazidime pentahydrate	Viscous eye drops	Spherisorb hexyl RP 5 μm (10 cm × 4.6 mm i.d.)	Acetonitrile/0.05 M ammonium acetate pH 7 (7:93 v/v)	UV 254 nm	Pyridine	[209]
15	Ciprofloxacin	Tablets	Lichrospher RP-18, 5 μm (25 cm × 4.6 mm i.d.)	Methanol/0.245% H_3PO_4 (3:22 v/v)	UV 278 nm	Potential impurities	[192]
16	Clindamycin	Tablets	Hypersil ODS (25 × 4.6 mm i.d.) 5 μm	Acetonitrile/phosphate buffer (pH 6)/water (7:8:5 v/v/v)	UV 210 nm	7-Epiclindamycin, clindamycin B, lincomycin, lincomycin-B and 7-epilincomycin	[68]
17	Dipyridamole	Injection	Waters μ-Bondapak C_{18} 10 μm (30 cm × 3.9 mm i.d.)	Aqueous sodium acetate pH 5/36% acetic acid/methanol	UV 276 nm	Degradation products	[205]
18	Erythromycin	Hydrophilic cream	PLRP-S (25 cm × 4.6 mm i.d.) 8 μm	2 methyl-2-propanol/acetonitrile/0.2 M phosphate buffer pH 11/H_2O (33:6:10:151 v/v/v/v)	UV 215 nm	Degradation products	[199]
19	Erythromycin	Tablets capsules	ODS—2, 5 μm (25 cm × 4.6 mm i.d.)	1:1 mixture of 10% acetonitrile in 0.2 M ammonium phosphate buffer of pH 6.5/0.2 M tetrabutyl ammonium sulfate of pH 6.5/H_2O (6:6:25 v/v/v–A) and 50% acetonitrile in the same solution as above-B	UV 205 nm	Related impurities	[202]
20	Fentanyl Citrate	Injection	C_8 Inertsil 5 μm (25 cm × 4.6 mm i.d.)	0.23% perchloric acid/acetonitrile (13:7 v/v)	UV 206 nm	1-Phenethyl-*N*-phenylpiperidin-4-amine	[189]
21	Fluxetine	Capsules	Zorbax SB-C_8 5 μm (25 cm × 4.6 mm i.d.)	Water/acetonitrile/TFA (8000:2000:7 v/v/v)	–	Impurities	[8]
22	Ipratropium bromide	Inhalations	Alltima C_{18}, 5 μm (25 cm × 4.6 mm i.d.)	100 mM KH_2PO_4/acetonitrile (4:1)-A, (11:9)-B	UV 210 nm	Tropic acid, *N*-isopropylnoratropine, 8-*S*-tropic acid and apo-ipratropium	[193]
23	Isoxsuprine	Tablets	μ-Bondapak C_{18} 10 μm (15 cm × 3.9 mm i.d.)	acetonitrile/potassium dihydrogen phosphate (0.01 M) pH 2.2 with H_3PO_4 (9:41 v/v)	UV 275 nm	4-Hydroxy benzaldehyde, 4-hydroxybenzylalcohol, 4-hydroxy benzoic acid and 4-hydroxy acetophenone	[99]

Table 3 (*Continued*)

S. No	Drug	Formulations	Column	Mobile phase	Detector	Impurities	Refs.
24	Ketokonazole	Tablets, cream, shampoo	Hypersil ODS (25 × 4.6 mm i.d.) 3 μm	Acetonitrile /20 mM Na_2HPO_4 pH 4 containing 0.2% diethylamine (3:2 v/v)	UV 232 nm	Related impurities	[190]
25	Lorazepam	Tablets	Wakosil 5 μm	0.1 M ammonium acetate (pH 6 with acetic acid)/acetonitrile/methanol (1:1:1 v/v/v)		Related compounds	[100]
26	Nalbuphine HCl	Injection	Zorbax SB-C_8 (15 cm × 4.6 mm i.d.) 5 μm	0.5% TFA in H_2O/acetonitrile (97:3)-A; 0.05% TFA in H_2O/THF/acetonitrile (72:25:3 v/v/v)-B	UV 280 nm	Degradation products	[195]
27	Nabumetone	Tablets	ODS, Zorbax (25 cm × 4.6 mm i.d.)	Aqueous 60% methanol	UV 261 nm	4-(6-Methoxy-2-napthyl) 4-hydroxybut-3-ene-1,2-dione and 2-acetyl 6-hydroxy napthalene	[58]
28	Oxytocin acetate	Injection	ODS, 5 μm (15 cm × 4.6 mm i.d.)	100 mM sodium phosphate monobasic of pH 3.1–4.5 and acetonitrile/H_2O (1:1 v/v)	UV 220 nm	Degradation products	[203]
29	Paclitaxel	Injections	Phenomenex cursosil PFP and of Whatman TAC-1, 5 μm (25 cm × 4.6 mm i.d.)	Water and acetonitrile deacetyl taxol gradient	UV 230 nm	Related taxanes	[94]
30	Pilocarpine	Ophthalmic solution	YMC-pack ODS-AM 5 μm	Methanol/H_3PO_4/triethylamine pH 3 (15 cm × 4.6 mm i.d.)	UV 214 nm	Degradation products	[200]
31	Ramipril	Altace capsules	Zorbax SB-C_{18} 3.5 μm (15 cm × 4.6 mm i.d.)	Acetonitrile and aq. buffer (0.025 M potassium phosphate and 0.0569 sodiumperchlorate) 27:73 v/v Acetonitrile and aq. buffer (0.025 M potassium phosphate and 0.014 M sodium perchlorate) 38:62 v/v pH 2.0 gradient	UV 210 nm	Related Substances	[49]
32	Ramipril and hydrochlorothiazide	Tablets	Supelcosil LC-8 5 μm (15 cm × 4.6 mm i.d.)	Acetonitrile/sodium perchlorate (0.1 M) adj. to pH 2.5 with H_3PO_4(46:54 v/v)	UV 210 nm	Degradation products	[215]
33	Riboavirin	Capsules and syrups	μ-Bondapak C_{18} (30 cm × 3.9 mm i.d.)	10 mM KH_2PO_4 pH 4.6 /methanol (19:1 v/v)	UV 207 nm	Triazole carboxamide, triazole carboxylic acid and ribose triazole carboxylic acid	[213]
34	Rifampicin	Capsules	Novapak C_{18}	0.02 M Na_2HPO_4/acetonitrile (13:7) pH 4.5 with H_3PO_4	UV 254 nm	Degradation products	[196]
35	Sunepitron	Tablets	Puresil C_{18} (15 cm × 4.6 mm i.d.)	Acetonitrile/water/aq. 0.05 M ammonium acetate pH 4.6 (6:3:91 v/v/v)	UV 238 nm	Potential impurities	[98]

Table 3 (*Continued*)

S. No	Drug	Formulations	Column	Mobile phase	Detector	Impurities	Refs.
36	Tamoxifen hydrogen citrate	Tablets	Ultra Sep ES Pharm RP 8, 4 μm (12.5 cm × 4 mm i.d.)	10 mM H_3PO_4 and 10 mM triethylamine buffer of pH 7.3/acetonitrile (3:22 v/v)	UV 270 nm	Impurities	[95]
37	Taxol	Injection	Fluofix (15 cm × 4.6 mm i.d.)	42 mM ammonium acetate/acetonitrile (3:2 v/v) at pH 5	UV 250 nm	10-Deacetyl baccatin III, baccatin III, 10-deacetyl taxol, cephalomannine plus 7-epi-10-	[96]
38	Tobramycin	Ophthalmic suspension	Novapak C_{18} (15 cm × 3.9 mm i.d.)	Acetonitrile/buffer (11:9 v/v) buffer:Tris/H_2O/(11:9 v/v) buffer:-Tris/H_2O/1 N H_2SO_4/acetonitrile	UV 365 nm	Neaamine, kanamycin and nebramine	[70]
39	Ursodeoxycholic acid	Capsules, tablets	Adsorbosphere 3 μm (10 cm × 4.6 mm i.d.)	Methanol/acetonitrile/0.1 M sodium acetate buffer of pH 6.5 (3:1:1 v/v/v)	Electrochemical Porus graphite electrode	Chenodeoxycholic acid	[207]
40	Vindesine	Injection	Alltima C_{18} 5 μm (15 cm × 4.6 mm i.d.)	Methanol/15% diethylamine pH 7.5 with H_3PO_4	UV 267 nm	Impurities	[91]
41	Vindesine sulfate	Injection	Kromasil C_{18} 5 μm (25 cm × 4.6 mm i.d.)	Aqueous 65% methanol pH 6.8 with H_3PO_4	UV 267 nm	Impurities	[198]
42	Vinpocetine	Tablets	Y or G-C_{18} H_{37} (25 cm × 4.6 mm i.d.)	Methanol/1.57 mM ammonium carbonate/diethyl ether	UV 274 nm	Methyl apovincamin-22-oate	[214]
43	Vorazole	Tablets	Hypersil/BDS C_{18} 3 μm (10 cm × 4.6 mm i.d.)	0.01 M tetra butyl ammonium hydrogen sulfate/acetonitrile pH 2.1–2.3	UV 230 nm	Related compounds	[197]
44	Zopiclone	Tablets	Lichrospher-60 RP Select B 5 μm (12.5 cm × 4 mm id)	18.06 mM monosodium hexane sulfonate/18 mM dihydrogen phosphate buffer pH 4.55/acetonitrile/THF (81:18:1 v/v/v)	UV 303 nm	Degradation products	[212]

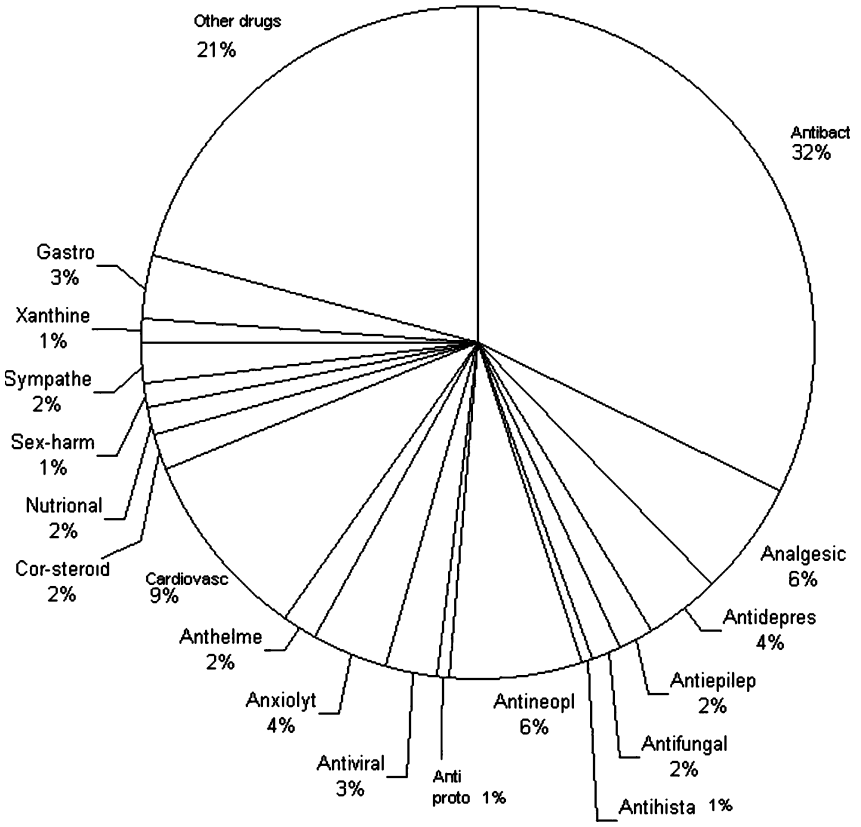


Fig. 7. Classification (based on analytical abstracts) of drugs analysed by HPLC during 1995–2001.

quantitation. It was used for investigating the impurities in several batches of pholcodine produced by different manufacturing units. Miller et al. [56] developed a simple LC method to limit the related substances like 2,3-dimethyl aniline in mefnamic acid using a 5 μm Nucleosil C_{18} column (25 cm × 4.6 mm i.d.) and mobile phase consisting of acetonitrile:water (45:55 v/v) (adjusted with phosphoric acid to pH 3.0) and detection at 210 nm. The limit of quantification of 2,3-dimethyl aniline was found to be 0.2 mg/ml. In one account, questions about the stability of aspirin have been addressed using HPLC. Aspirin and its degradation products viz., salicylic acid, acetyl salicylic acid and salicyl salicylic acid were determined in a model aerosol using a C_8 Econosphere column (5 μm, 25 cm × 4.6 mm i.d.) and MeOH:THF:1 M-H_3PO_4:H_2O (44:5:5:46 v/v/v/v) as mobile phase and UV detector set at 275 nm [57]. Nabumetone and two of its impurities viz., 4-(6-methoxy-2-napthyl)-4-hydroxybut-3-ene-1,2-dione and 2-acetyl-6-hydroxy napthalene were separated and determined quantitatively using a 5 μm Zorbax ODS column (25 cm × 4.6 mm i.d.) with aqueous 60% methanol as mobile phase and detection at 261 nm [58]. Several RP-HPLC methods for determination of process-related impurities of celecoxib [59], rocicoxib [60] and oxaprozin [61] belonging to non-steroidal anti-inflammatory drugs were developed and validated.

Formulations have received considerable attention during this period. Degradation products of beclomethasone dipropionate were separated, identified and quantified in one of its formulations using Lichrosorb RP-18 column (25 cm × 4.6 mm i.d., 10 μm) with aqueous acetonitrile as mobile phase and detection at 239 nm using gradient RP-HPLC [54]. Tablets of acetaminophen were sub-

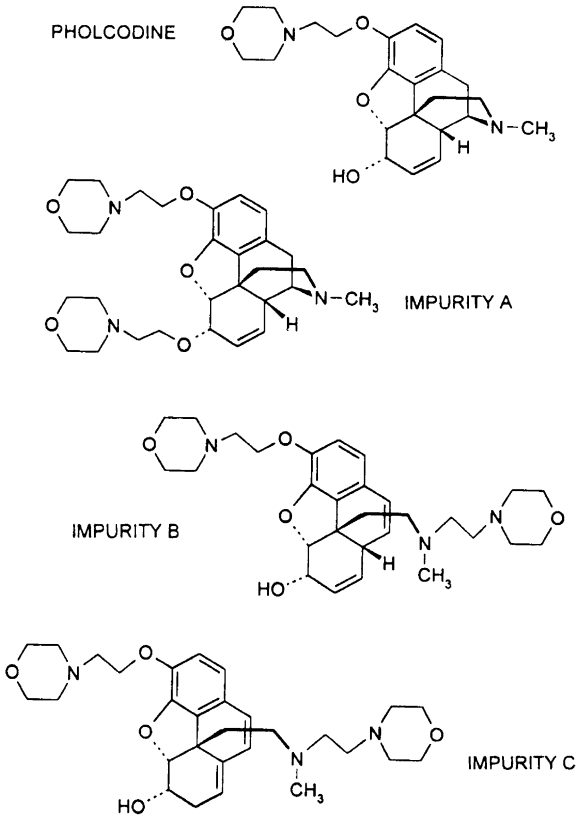


Fig. 8. Structural formulae of pholcodine and the isolated impurities (A, B and C).

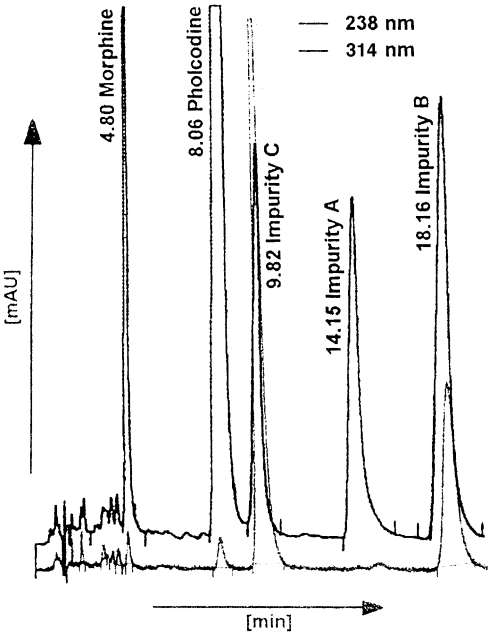


Fig. 9. HPLC chromatograms of standard mixture of morphine, Pholcodine and impurities A, B and C separated on a Luna phenyl–hexyl 5 μ column (15 cm × 4.6 mm i.d.). The mobile phase consisted of 10% (v/v) acetonitrile, 7% (v/v) in 20 mM phospahte buffer (pH 8.0) delivered at a flow-rate of 2 ml/min. Detection: 238 and 314 nm.

jected to RP-HPLC system for determination of degradation products viz., *O*-acetyl acetaminophen, *O*-ethylacetaminophen, *O*-(2-nitrobenzenesulfonyl)ace-taminophen, *O*-benzylacetaminophen and *O*-(3,5-dinitro benzyl) acetaminophen [53]. 4-Acetyl amino phenyl acetic acid known as Actarit was separated from its related compounds viz., 4-aminophenylacetic acid, 4-nitrophenylacetonitrile and 4-nitrophenylacetic acid using Kromsil C_{18} column (5 μm, 25 cm × 4.6 mm i.d.) with a mobile phase containing 70% methanol and 1% tetra butyl ammonium bromide in water flowing 1 ml/min with a UV detection at 245 nm [62].

3.2. Antibacterial and antimycobacterial agents

Many of the methods used for analysis of antibiotics continued to be based on HPLC. The most common approach used was the relatively simple isocratic reversed-phase in combination with UV detection. For example, Hess et al. [63] have developed and validated a rapid and sensitive LC-UV method for identification of new impurities which could not be detected by known methods of EP and USP. Now a days, manufactures all over the world are switching over to the synthesis of drugs not protected by patents by introducing procedures more or less different from the original [64]. One such example is the well known catastrophe based on undetected impurities of L-tryptophan which were found to be responsible for severe eosinophilia–myalgia syndrome [65]. In light of this, the work of Hess et al. has become important and emphasizes the necessity to update the analytical methods of widely used drugs whose manufacturing procedures are changed from time to time. Further, the investigations of Hess et al. have led to the revision of monograph of trimethoprim in the EP [66]. The impurities viz., diveridine, 2,4-

diamino-5-(4-ethoxy-3,5-dimethoxybenzyl)pyrimidine, 2,4-diamino-5-(3,4,5-trimethoxybenzoyl)pyrimidine, 4-amino-2-methylamino-5-(3,4,5-trimethoxybenzyl)pyrimidine, 2-amino-4-methyl-amino-5-(3,4,5-trimethoxybenzyl)pyrimidine and 2,4-diamino-5-(3-bromo-4,5-dimethoxobenzyl)pyrimidine were separated on a Nucleosil 120-5, C_{18} AB (25 cm × 4.6 mm i.d.) column with a mobile phase consisting of methanol/aqueous solution of sodium perchlorate monohydrate (3:7 v/v) adjusted to pH 3.0 with phosphoric acid and detection at 280 nm and 254 nm. Other examples of many procedures published for the analysis of antibiotics include the simultaneous determination of impurities of pseudoephedrine HCl and naproxen using a 5 μm Waters Sperisorb cyano column (25 cm × 4.6 mm i.d.). An isocratic elution was carried out with a mixture of triethylamine/methanol/water (1:15:15:170 v/v/v) adjusted to pH 3.7 with formic acid as mobile phase. The UV detector was set at 260 nm and switched to 235 nm before the elution of 2-ethyl-6-methoxynaphthalene. Impurities such as benzaldehyde, benzoic acid and 2-(methylamino)-propiophenone HCl of pseudoephedrine HCl as well as 2-(6′-hydroxy-2′-napthyl)propionic acid, 2-hydroxy-6-methoxy-napthalene,1-(6′-methoxy-2′-napthyl)ethanol,2-acetyl-6-methoxynapthalene and 2-ethyl-6-methoxynaphthalene of naproxen were separated [67]. Orwa et al. have developed a method for separation of clindamycin and its related substances viz., 7-epiclindamycin, clindamycin B, lincomycin, lincomycin B and 7-epilincomycin on a 5 μm Hypersil ODS column (25 cm × 4.6 mm i.d.) operating at 45 °C with acetonitrile/phosphate buffer (1.35% phosphoric acid adjusted to pH 6 with ammonia)/water (7:8:5 v/v/v) as mobile phase and detection at 210 nm [68]. An inter-laboratory study on determination of impurities in dicloxacillin sodium was reported during this period [69]. It involved separation of related impurities of dicloxacillin on ODS column (25 cm × 4.6 mm i.d.) with 0.27% phosphate buffer (adjusted to pH 5 with NaOH)/acetonitrile (3:1 v/v) as mobile phase and detection at 225 nm. A modified USP method proposed by Russ et al. was used to determine tobramycin and its related substances viz., namine, kanamycin and a degradation product, nebramine in an opthalmic ointment treated with 0.8 mM-H_2SO_4 at 70 °C for 20 min [70]. The derivatives were analyzed on a Nova-pak C_{18} column (25 cm × 4.6 mm i.d.) with 11:9 (v/v) acetonitrile/buffer (containing tris/water/1 N H_2SO_4/acetonitrile) as mobile phase and detection at 365 nm. An ion-pair RP-HPLC method was developed for separation and determination of ceftriaxone and its related impurities viz., 7-aminocephalo-sporanic acid and 7-amino-3-[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-1,2,4-triazin-3-yl) thiomethyl-3-cephem-4-carboxylic acid on a 5 μm Kromasil C_{18} column (20 cm × 4.6 mm i.d.) with acetonitrile/tetrabutyl ammonium bromide/phosphate buffer of pH 7/water (400:4:55:795 v/v/v) as mobile phase at 1 ml/min and detection at 270 nm [71]. Lincomycin and its related substances viz., lincomycin B and 7-epilincomycin, as well as three unidentified minor impurities were separated on a column (25 cm × 4.6 mm i.d.) of base deactivated 5 μm Supelcosil LC-ABZ C_{12}–C_{18} at 45 °C with a mobile phase of 2.25% acetonitrile, 2.72% KH_2PO_4 adjusted to pH 5 with 3.48% KH_2PO_4 and 0.067% methane sulfonic acid in water and detection at 210 nm [72]. Ampicillin, cloxacillin and their related substances were determined on a 3 μm ODS-Hypersil column (10 cm × 2 mm i.d.) with acetonitrile/20 mM-phosphate buffer of pH 2 (15:85 v/v) containing 100 mM-sodium dodecyl sulfate (SDS) as mobile phase and detection at 230 nm. [73]. Cefdinir and its related substances were determined on a 5 μm TSK gel ODS—80 TM column (7.5 cm × 4.6 mm i.d.) operated at 25 °C with 33 mM-citrate-phosphate buffer of pH 2/methanol/dioxane (36:4:1 v/v/v) as mobile phase and detection at 254 nm [74].

Ciprofloxacin, an example from our own work has demonstrated the usefulness of HPLC for monitoring process impurities during the production [75]. Various reactions involved in the manufacture of ciprofloxacin are shown in Fig. 10. During its production a large number intermediates viz., dichlorofluroacetophenone, cyclopropylamine, chlorofluoroaniline, cyclopropyl acrylate, quinolonic acid and piperazine were synthesized and ultimately converted to ciprofloxacin. A reversed-phase C_{18} column Flexit ODS (30 cm ×

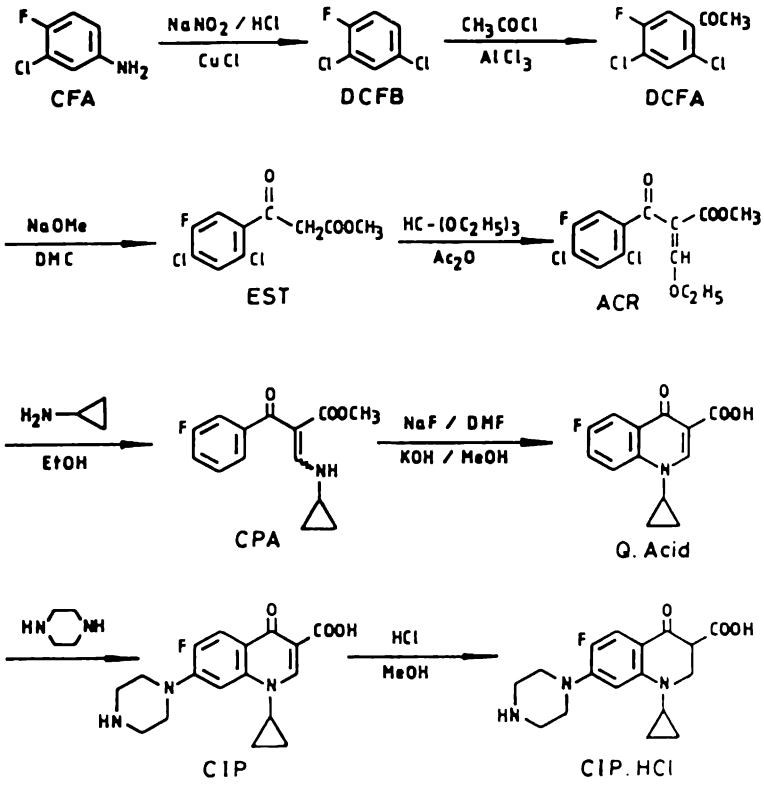


Fig. 10. Reaction involved in the preparation of ciprofloxacin hydrochloride.

4.6 mm i.d.) with water–methanol–acetic acid (84:15.9:0.1 v/v/v) as eluent and detection at 254 nm was used to separate the related impurities and detect them in the finished products (Fig. 11). The use of evaporative light scattering detector for determination of isepamicin sulfate and related compounds by HPLC has been reported. Isepamicin sulfate belongs to a class of aminoglycoside antibiotics and lacks a suitable chromophore for UV detection. For this reason it is usually analyzed by using pre or post column derivatization. However these methods required labour intensive sample preparation and contain salt laden mobile phases, which are abusive to pumps and injectors of HPLC systems. Detection using refractive-index is restricted because of significant base line drifts effecting accuracy and detectability of the analytes. The method developed by Vogel et al. enabled the separation of isepamicin from its geometric isomers bisepamycin and other related compounds which are lacking UV chromophores. The separation was achieved using two Waters X-Terra RP-18 columns connected in tandem at 10 °C [76].

3.3. Anti-viral, antifungal and anthelmintic drugs

HPLC has played a major role in the analysis of this major class of compounds. In one instant, Montgomery et al. have reported the development and validation of a reversed-phase stability indicating method for analysis of efavirenz and it related substances in bulk drug and formulations [48]. Efavirenz is a non-nucleoside inhibitor used in the treatment of HIV-1 reverse transcriptase. It is used in combination with other anti-retroviral agents for the treatment of HIV-1 infection in children and adults. Currently, efavirenz is marketed in 50, 100 and 200 mg strength capsules. The structures of efavirenz and its related substances

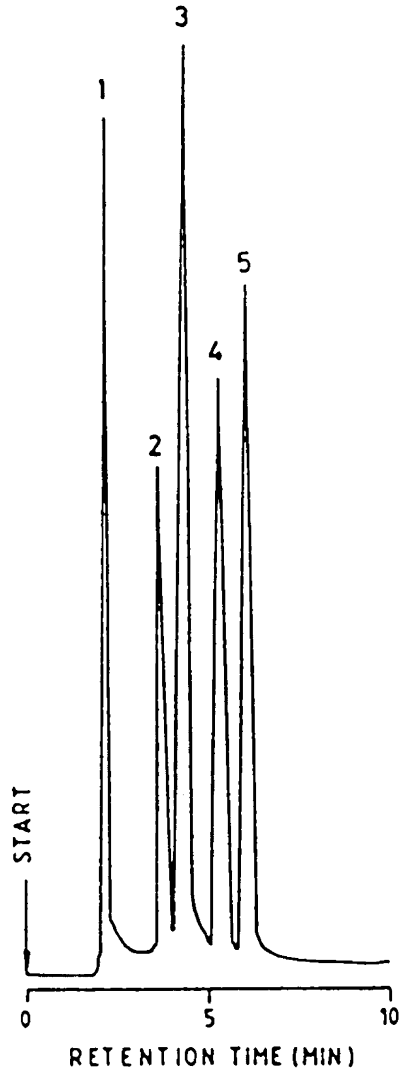


Fig. 11. HPLC chromatogram of a mixture containing ciprofloxacin and its components (1) CIP, (2) DCFA, (3) Q-acid, (4) CPA and (5) CFA.

are shown in Fig. 12. The drug and the impurities viz., methyl DMP 266 SV 997, SD 573-methyl carbamate SV 993, SD 572, SG 275 and SW 965 were separated and determined on a Zorbax SB-CN column (15 cm × 4.6 mm i.d.) at 60 °C with a gradient mobile phase (A) 90% water, with 0.05% trifluoroacetic acid, 10% methanol (B) 90% methanol, 10% water, with 0.05% trifluroacetic acid with a UV detection at 250 nm (Fig. 13). This method was validated as per ICH guidelines. Another report dealing with an improved RP-LC method for determination of acyclovir using a Spherisorb ODS (25 cm × 4.6 mm i.d.) analytical column with methanol/5 mM-monopotassium dihydrogen phosphate buffer of pH 3.0 (1:19) as mobile phase pumped isocratically at 1.3 ml/min and UV detection at 254 nm was reported [77]. Nevirapine and its related impurities were studied for their separation and determination on a Supelcosil LC-ABZ column using a mobile phase consisting of acetonitrile/25 mM ammonium dihydrogen phosphate (pH 5) (1:4 v/v) and UV detection at a wavelength of 220 nm [78]. Tolnaftate and related impurities viz., *N*-methyl-*m*-toluidine and β-naphthol-1-chlorothiocarbamate were determined on a 5 μm Hypersil-phenyl column (25 cm × 4.6 mm i.d.) with water/methanol (2:3 v/v) as mobile phase and detection at 250 nm [79]. One of the authors along with Gomes studied anthelmintic drugs viz., mebendazole, fenbendazole and albendazole (for structures see Fig. 14) for separation and determination of their potential impurities by HPLC [80]. A reversed-phase Inertsil C_{18} (GL Sciences Inc., Tokyo, Japan) column (25 × 4.6 mm i.d. particle size 5 μm) was used for the separation of all the three products and their impurities. The mobile phase for mebendazole was a mixture of orthophosphoric acid (0.1% v/v in H_2O) and acetonitrile (70:30 v/v) pH adjusted to 6.0 with 1 M potassium hydroxide solution, whereas for fenbendazole it was orthophosphoric acid (0.1% v/v in H_2O) and acetonitrile (40:60 v/v), pH adjusted to 4.0 with 1 M potassium hydroxide. Methanol–water–perchloric acid (45:55:0.3 v/v/v) was used as mobile phase for albendazole. Chromatograms shown in Fig. 15 were recorded at their respective absorption maxima i.e. 290 nm for both mebendazole and fenbendazole, 254 nm for albendazole using a UV detector.

3.4. Cardiovascular and anti-hypertensive agents

Methods based on gradient reversed-phase conditions are found to be very popular for analyzing these drugs. During the period of review, as many as 20 methods were published, out of which most of the methods are based on gradient elution

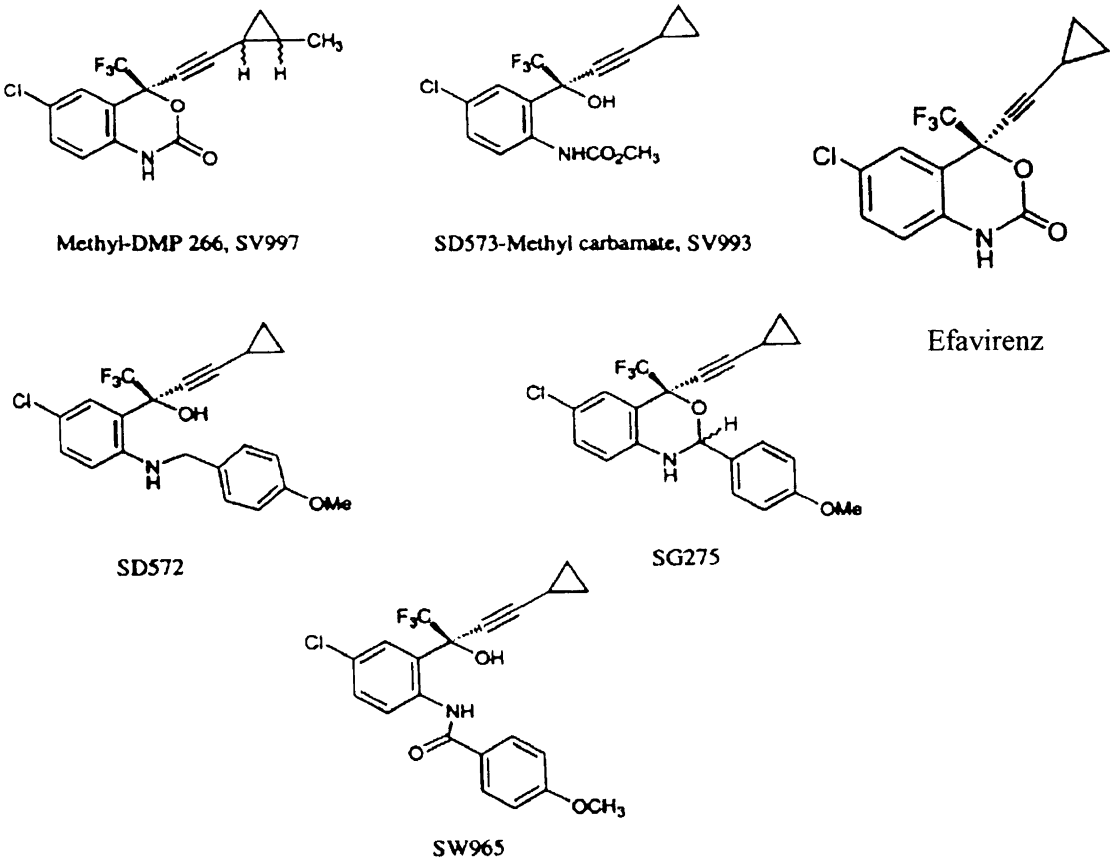


Fig. 12. Chemical structures of efavirenz potential synthetic impurities and degradation products.

techniques for analyzing anti-hypertensive drugs. However the isocratic conditions for separation of nitrendipine and its impurities of reaction partners and by-products (methyl-3-amino crotonate, 2-ethyl-2-(nitro-benzylidene) acetoacetate, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid diethyl ester and 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)3,5-pyridine dicarboxylic acid dimethyl ester) on a Lichrosorb RP-18 5 μm (25 cm × 4 mm i.d.) methanol–water (70:30) as adjusted to pH 3 with H_3PO_4 and detection at 238 nm were also reported [81]. Ramipril [CAS No. 8733-19-5] is one of the several angiotensin-converting enzyme inhibitors which is used as a prodrug for regulating of hypertension. The chemical structures of ramipril and its related substances are shown in Fig. 16. The structure of ramipril is to similar to that of a proline containing natural peptide. Till 1999, methods describing the determination of related substances of ramipril are not listed in USP or EP. Quite recently, Hogan et al. have developed and validated a reversed-phase HPLC method for separation of related substances of ramipril in Altace capsules using an ion-pairing reagent and a simple two step gradient in a 40 min run time [49]. The chromatograms are shown in Fig. 17. In another attempt, as many as 13 impurities were separated from verapamil HCl on a 5 μm Supelco pK_b-100 column (25 cm × 4 mm i.d.) with gradient elution using 0.04 M-dibasic potassium phosphate at pH 7.2 and acetonitrile as mobile phase. The calibration graphs were linear in the range of 0.01–1.0% of each impurity, the detection limits were ∼0.05 μg/ml and the quantitation limits were ∼1 μg/ml [82]. A gradient liquid chromatographic (LC) stability

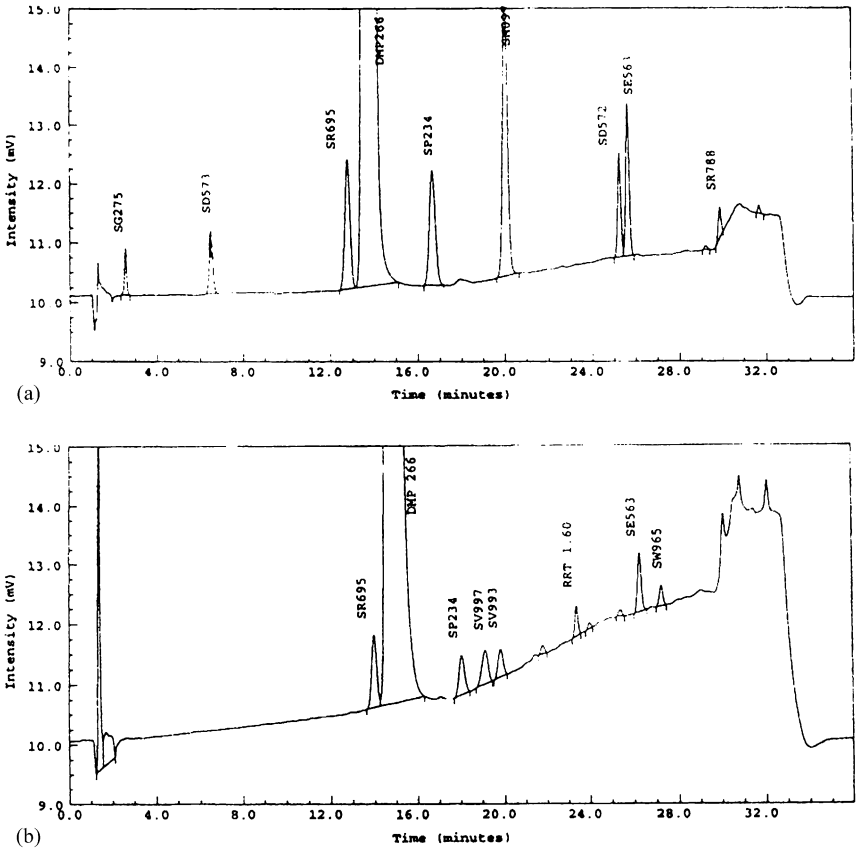


Fig. 13. (a) Chromatogram of efavirinz drug substance spiked with its known potential synthetic impurities (at 0.5%), (b) chromotogram of efavirenz drug substance containing additional related substances (SR 695 is present at 0.13%).

indicating method for evaluation of purity of benazepril hydrochloride in bulk drug and pharmaceutical dosage forms using 5-methyl-2-nitro phenol as internal standard was reported [83]. A 5 μm Select B RP-8 column (25 cm × 4 mm i.d.) with gradient elution using acetonitrile and water acidified to pH 3.0 with phosphoric acid and detection at 280 nm was used to separate and determine 4-hydroxy coumarin, 4-nitrobenzalacetone and the major synthetic impurities of warfarin and acenocoumarol [84]. β-Methyldigoxin and its seven impurities were separated on a 10 μm Spherisorb C_{18} column (15 cm × 4 mm i.d.) with a mobile phase consisting of acetonitrile/water (17:33 v/v) at a flow rate of 1 ml/min and detection at 218 nm [85].

3.5. Gastro-intestinal agents

A stability indicating assay useful for the determination of impurities and degradants viz., [5-[[(2-aminoethyl)-thio]methyl]-*N*,*N*-dimethyl-2-furanmethanamine,(*N*,*N*′-bis[2-[[[5-[(dimethylamino)methyl]-2-furanyl]methyl]thio]ethyl]-2-nitro-1,1′-ethene diamine and ranitidine-*S*-oxide present in both bulk drug and finished products of ranitidine HCl was developed using a YMC-pack ODS-AM (15 cm × 4.6 mm i.d.) column and the mobile phase consisting of 10 mM SDS, 50 mM H_3PO_4 adjusted to pH 6.8 with TEA and 43% methanol [86]. The detection was at 228 nm and the column temperature was 35 °C. An ion-pair HPLC on a 10 μm Nucleosil C-18 column (25

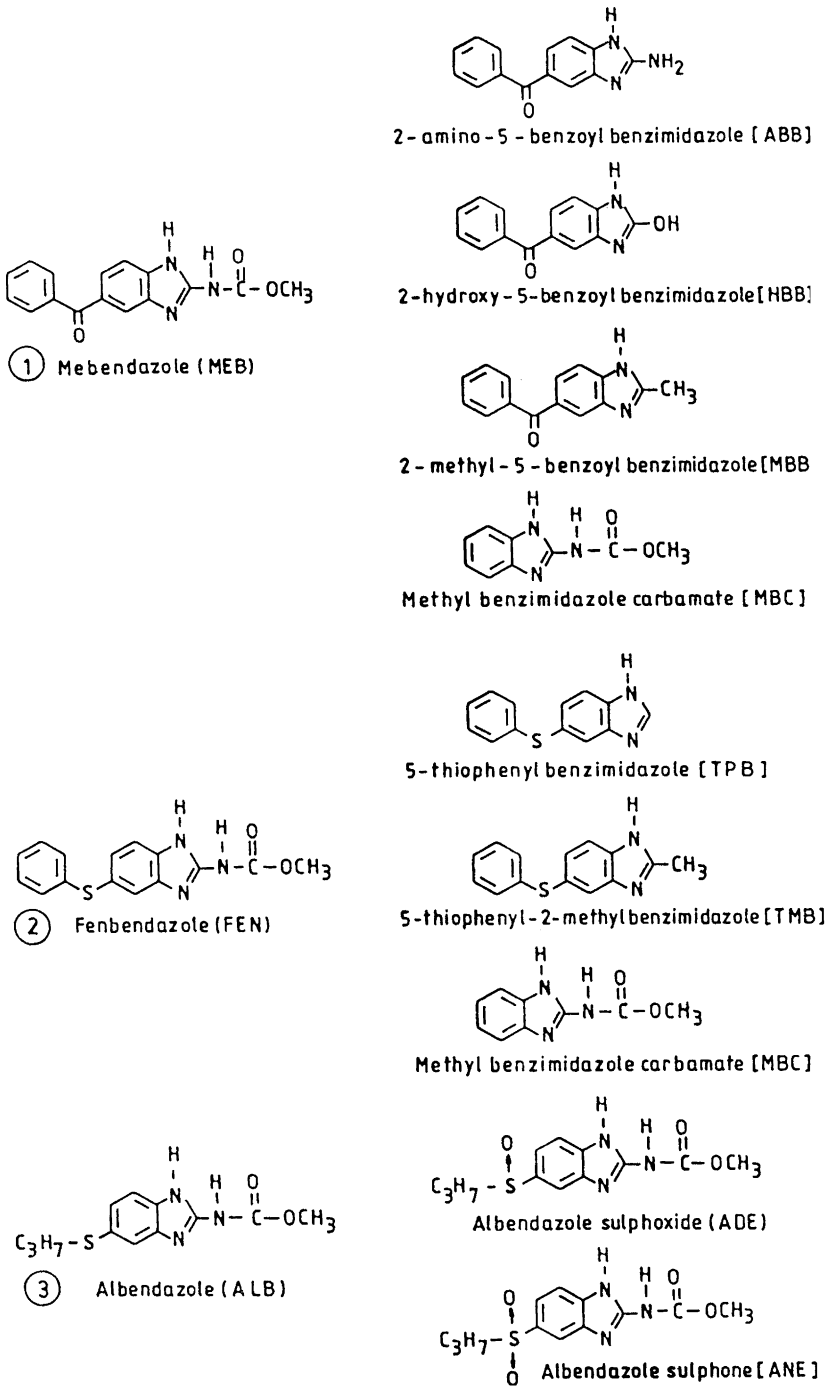


Fig. 14. Chemical structures of anthelmintic, mebendazole (1) fenbendazole (2) albendazole (3) and their process impurities.

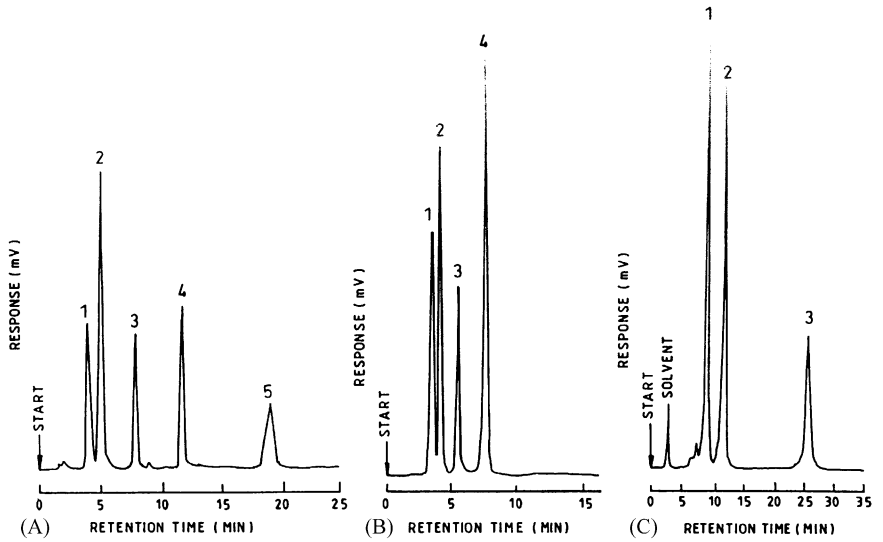
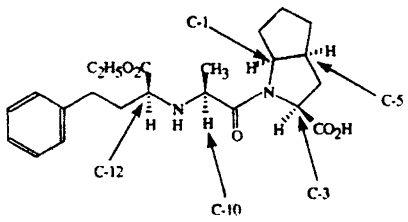


Fig. 15. (A) HPLC chromatogram of a mixture containing (1) ABB (2) MBC (3) HBB (4) MBB (5) MEB. (B) HPLC chromatogram of a mixture containing (1) MBC (2) TMB (3) TPB and (4) FEN. (C) HPLC chromatogram of a mixture containing (1) ADE (2) ANE and ALB.

cm × 4 mm i.d.) and the mobile phase prepared by mixing 270 ml methanol, 0.3 ml 85% phosphoric acid and 0.94 g sodium 1-hexane-sulfonate and diluting to 1 l with water and a diode array detector at 220 nm to separate several impurities of cimitidine was reported [87]. A simple and rapid HPLC method for separation and determination of process impurities of famotidine (for structures see Fig. 18) in bulk drug and formulations was developed by our own group [88]. A reversed-phase C_8 column with 0.01 M potassium dihydrogen phosphate–acetonitrile (75:25 v/v pH 3.15) as eluent was used at ambient temperature and the detection was at 254 nm. The HPLC chromatogram of a synthetic mixture containing all the process components viz., thiourea, chloro-*N*-(aminosulfonyl)propanimidoamine, diaminomethyleneamino(chloromethyl)thiozole and diaminomethyleneamino(1-amino-1′-iminomethelene)thiomethyl thiazole of famotidine is shown in Fig. 19.

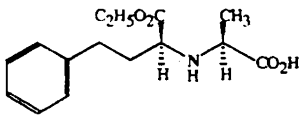
3.6. Anti-neoplastics and immunosuppressants

A standard addition technique was applied to quantify the levels of fluoride in technical leuprolide using RP-LC [89]. The method was based on formation of the ternary fluoride complex with zirconium (IV) and 2-(5-bromo-2-pyridylazo)-5-diethyl aminophenol. The analysis was performed on a Nucleosil C_{18} column with Lichrosphere end-capped RP-100 phase, using acetonitrile:water (85:15 v/v) at pH 4.0 as an eluent. The results were found to be comparable with those obtained by spectrophotometry. A triangular optimization technique was adopted to determine the optimum mobile phase composition for separation of finasteride from the products formed during its synthesis [90]. HPLC was performed on a Supelcosil LC-18 DB column (25 cm × 4.6 mm i.d.) with a mobile phase at a flow rate of 1 ml/min and the detection at 210 nm. The optimum mobile phase composition was found to be THF/methanol/TFA/water (30:5:0.025:65 v/v/v/v). Vindesine sulfate and its four impurities were well separated and determined in bulk and injections on a 5 μm Alltima C_{18} column (15 cm × 4.6 mm i.d.) with methanol/15% diethylamine adjusted to pH 7.5 with phosphoric acid (7:3 v/v) as mobile phase and detection at 267 nm. The tailing factor of the vindesine was 1–1.05 [91]. A gradient HPLC method for the determination of melphalan and

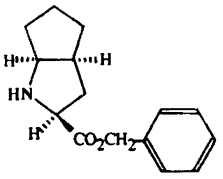


(a)

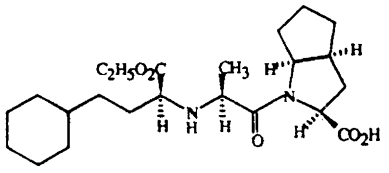
Diastereomer Abbreviation	Configuration at Listed Carbon Center				
	1	3	5	10	12
SSSSS (Ramipril)	S	S	S	S	S
SRSSS	S	R	S	S	S
SSSRS	S	S	S	R	S
RRRSS	R	R	R	S	S
SSSSR	S	S	S	S	R



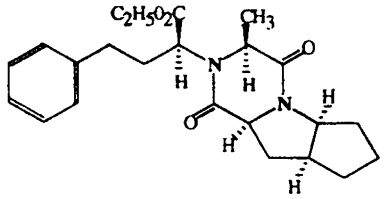
Precursor I



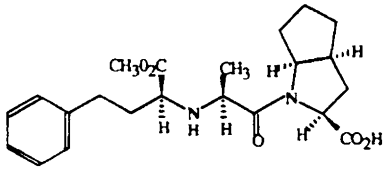
Precursor II-8



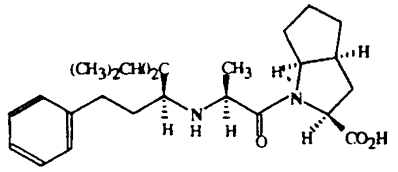
Hexahydro-ramipril



Ramipril-Diketopiperazine



(b) Ramipril Methyl Ester



Ramipril Isopropyl Ester

Fig. 16. (a) Chemical structure of ramipril and four potential related substances (ramipril diastereomers). (b) Chemical structure of six additional relevant related substances of ramipril.

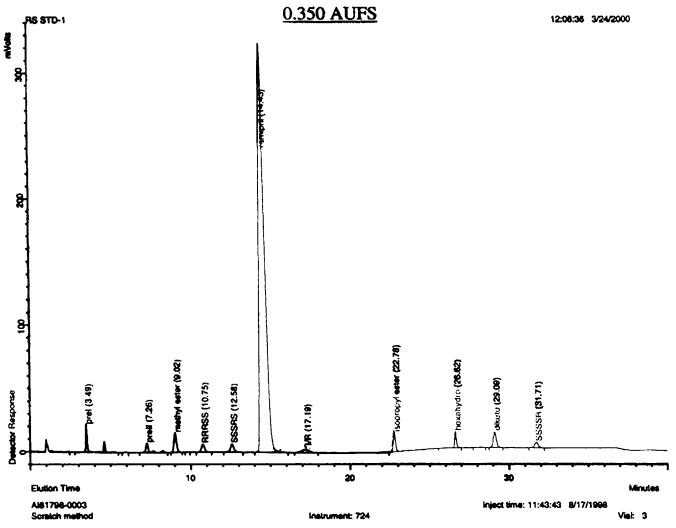


Fig. 17. HPLC chromatogram of ramipril and its related substances.

related impurities was reported by Brightman et al. [92]. A 5 μm BDS Hypersil C_{18} column (15 cm × 4.6 mm i.d.) with a mobile phase consisting of (acetic acid/triethyl amine/ammonium acetate/water; 5:1:5:44 v/v/v/v) /acetonitrile/water (1:120:80 v/v/v) and detection at 260 nm was used. Azathioprine and its intermediates viz., 6-mercaptopurine and 1-methyl-4-nitroimidazole were separated and determined on 5 μm Shimpack CLC-ODS column (15 cm × 4.6 mm i.d.) with a mobile phase (aqueous 30% acetonitrile) flowing at 0.8 ml/min and detection at 210 nm [93]. Two novel pentafluorophenyl (PFP) stationary phases along with four different aqueous acetonitrile solvent systems were used to separate and determine paclitaxel and its 15 related taxanes by HPLC. Columns (25 cm × 4.6 mm i.d.) of Phenomenex curosil PFP and of Whatman TAC-1 (both 5 μm) were used with mobile phase of water and acetonitrile in gradient elution and detection at 230 nm [94]. Tablets of tamoxifen hydrogen citrate were analyzed on a base-selective 4 μm Ultra Sep ES pharm RP8 column (12.5 cm × 4 mm i.d.) operated at 35 °C with 10 mM-phosphoric acid and 10 mM-triethylamine buffer of pH 7.3/acetonitrile (3:22 v/v) as mobile phase and detection at 270 nm [95]. The method gave better separation not only of the Z and E isomers of tamoxifen but also the impurities of bis-tamoxifen and desmethyl-1-tamoxifen than the ion-pair chromatographic separation on a phenyl column following the procedures adopted by USP. Taxol and five of its related derivatives were also analyzed by LC using polyfluorinated RP column known as Flufix (15 cm × 4.6 mm i.d.) with 42 mM-ammonium acetate/acetonitrile (3:2 v/v) at pH 5 as the mobile phase (1 ml/min) and UV detection at 250 nm. The elution order was 10-deacetyl baccatin III, baccatin III, 10-deacetyl taxol, cephalomannine, 7-epi-10-deacetyl taxol and taxol. The separation was completed in less than 12 min [96].

3.7. Anti-depressants and antipsychotics

Nefazodone hydrochloride is an anti-depressant which acts both as a 5-hydroxytrytamine (5-HT) re-uptake inhibitor and a postsynaptic 5-HT2-receptor antagonist without cardio toxicity or sedative side effects. It increases prolactin levels and oral temperature by down regulation of 5-HTIC receptors or 5-HTIC receptor blockade. The clinical anti-depressant activity of nefazodone is attributed to the potentiation of the central serotonergic system. An Inertsil ODS-3V (25 cm × 4.6 mm i.d.) column with a mobile phase comprising 0.05 M KH_2PO_4 (pH 3.0), acetonitrile and methanol in the ratio 50:40:10 (v/v/v) was used

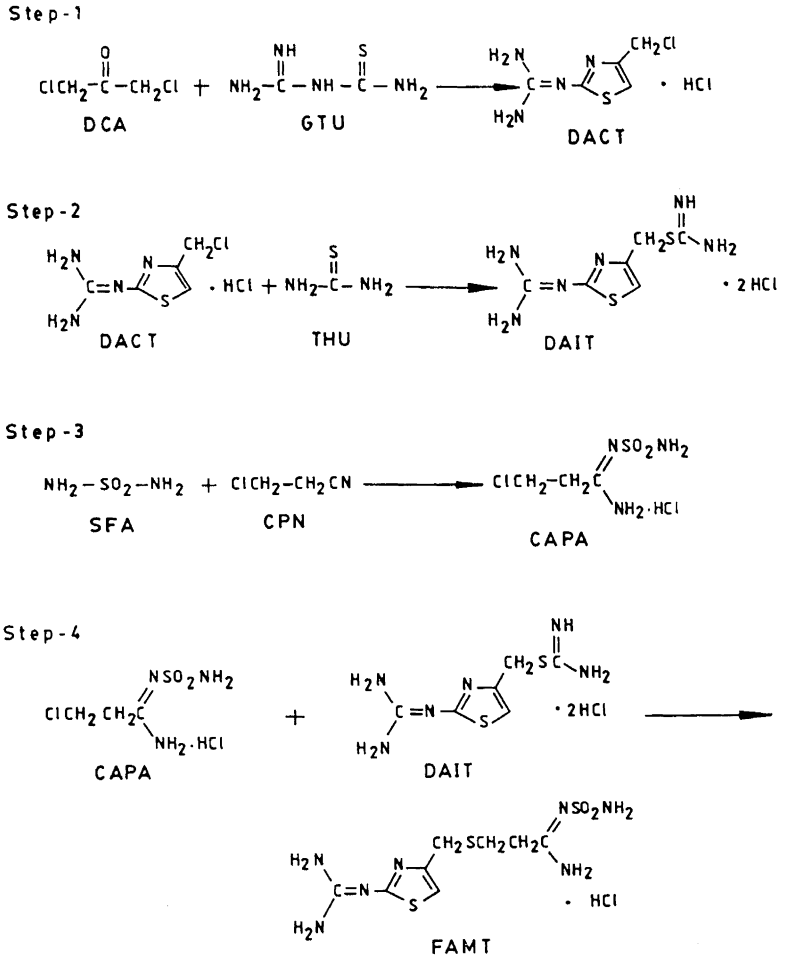


Fig. 18. Reaction involved in the preparation of famotidine hydrochloride.

to develop a simple, selective and reproducible reversed-phase LC method for the quantitative determination of nefazodone hydrochloride (I) in the presence of its related impurities, namely 5-ethyl-4-(2-phenoxyethyl)-2H-1,2,4-triazol-3-(4H)one (II), 1-(3-chlorophenyl)-4-(3-chloropropyl) piperazine hydro-chloride(III) and 1,1,1-trimethylene-bis[4-(3-chlorophenyl)piperazine] hydrochloride (IV) [97]. The method was completely validated and proved to be rugged. The limit of detection and quantitation for impurities II, III and IV were found to be 50, 79 and 91 ng/ml, and 152, 240 and 280 ng/ml, respectively. The intra- and inter-day assay precision of the method was within 1.2% R.S.D. The developed method was applied to the pharmaceutical dosage forms viz., tablet Serzone-R and the percentage recoveries ranged from 99.1 to 100.7. The percentage recovery of impurities ranged from 96.2 to 108.9. The stability studies were performed for nefazodone solution placed on laboratory bench and in the refrigerator for 60 days. The method was proved to be stability indicating in solution. Sunepitron and three of its potential impurities were determined on a 5 μm Puresil C_{18} column (15 cm × 4.6 mm i.d.), acetonitrile/water/aqueous 0.05 M ammonium acetate at pH 4.6 (6:3:91 v/v/v) as mobile phase and detection at 238 nm [98]. Degradation products of isoxsuprine viz., 4-hydroxy benzaldehyde, 4-hydroxybenzylalcohol, 4-

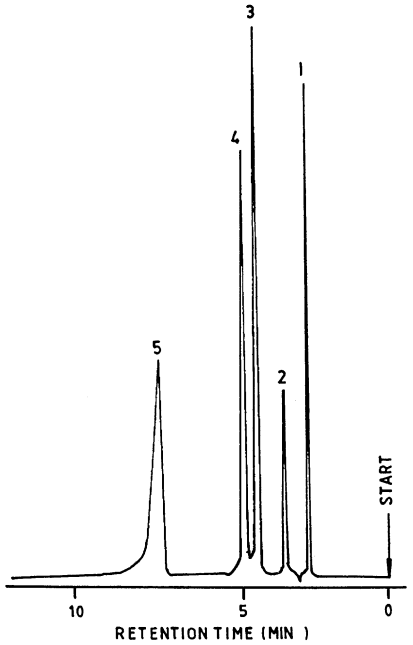


Fig. 19. Typical chromatogram of a mixture containing (1) THU, (2) CAPA, (3) DACT, (4) FAMT and (5) DAIT.

hydroxy benzoic acid and 4-hydroxy acetophenone were separated and determined on a μ-Bondapak C_{18} 10 μm column (15 cm × 3.9 mm i.d.) with a mobile phase consisting of acetonitrile/potassium dihydrogen phosphate (0.01 M) adjusted to pH 2.2 ± 0.1 with phosphoric acid (9:41 v/v) and UV detection at 275 nm [99]. Lorazepam and its related compounds were separated and determined on 5 μm Wakosil column (15 cm × 4.6 mm i.d.) with 0.1 M-ammonium acetate (pH adjusted to 6 with acetic acid)/acetonitrile/methanol (1:1:1 v/v/v) as mobile phase [100]. Twelve substances related to fluoxetine HCl were separated and determined on 3 μm nitrile (Spherisorb) column (15 cm × 4.6 mm i.d.) with THF/acetonitrile/0.25% TFA (1:3:16 v/v/v) as mobile phase and detection at 214 nm [101]. Later, a group from Eli-Lilly has studied the HPLC behavior of the same compounds on a 5 μm Zorbax SB-C8 column (25 cm × 4.6 mm i.d.) with water/acetonitrile/TFA (8000:2000:7 v/v/v) as mobile phase (1 ml/min) with linear gradient using GC and NMR simultaneously [8].

3.8. Antiepileptics

A non-porous-silica of 1.5 μm ODS-1 (3.3 cm × 4.6 mm i.d.) with buffer (3.42 g potassium dihydrogen phosphate in 500 ml water, 1 ml triethyl amine adjusted to pH 2.5 with 0.1 M phosphoric acid)/acetonitirle (77:23 v/v) as mobile phase and detection at 220 nm was used to determine haloperidol and (4,4′-bis [4-(*p*-chlorophenyl)-4-hydroxy piperidino]butyrophenone) [102]. The detection limits were 1 and 10 ng/ml for the drug and the impurity, respectively. The ion chromatographic method proposed by Micheel et al. detects and quantifies sulfamate and sulfate ions (inorganic part of decomposition) in topiramate and in conjunction with an assay method for topiramate and one of its known degradation products provided total molar accountability. The chromatographic system consisted of a NaOH gradient and an anion-exchange Dionex, Ion Pac AS5A-5 μm column (15 cm × 4.0 mm i.d.) and anion suppresser [103].

3.9. Other drugs

A wide variety of methodologies have appeared for other important classes of drugs including antiallergic, antiparkinsonian, insecticide and diagnostic aids. Known impurities viz., cromlyn diethyl ether and hydroxy phenoxy-2-propanol of cromlyn sodium were separated on Nova-pak C_8 column (15 cm × 3.9 mm i.d.) with methanol/1 M-tetrabutyl ammonium dihydrogen phosphate buffer (9:11 v/v) as mobile phase and detection at 326 nm [104]. The method was linear from 0.05–2% of the working range for impurities and 46–137% for assay. An experimental design to optimize the chromatographic conditions for separation of ropinirole and five of its synthetic impurities in a single run was described. Aqueous solutions were analyzed on a 5 μm Nucleosil 100-5 C_{18} μm (41 cm × 0.32 mm i.d.) operated with 8.7 m M-MES adjusted to pH 6.0 with NaOH/acetonitrile (9:11 v/v) as mobile phase and detection at 254 nm [105]. An evaporative light scattering detector connected

to a 3 μm Hypersil ODS-RP-18 column (10 cm × 2.1 mm i.d.) with methanol/acetonitrile/water (30:11:9 v/v/v) adjusted to pH 4, with glacial acetic acid as mobile phase was used to separate ursodeoxycholic acid (UDCA) and its related impurities viz., ursocholica acid, cholic acid, chenodeoxycholic acid deoxycholic acid and litocholic acid. Calibration graphs were linear in the range of 40–1120 μg/ml for UDCA and 50–560 μg/ml for the impurities [106]. An LC method was reported to separate diatrizoate sodium from its impurities viz., 2,4-and 2,6-di-iodo-3,5-diacetamidobenzoic acid. Separation was performed on a PRP-X100 column (10 cm × 4.1 mm i.d.) with 0.1 M KCl/0.05 M-dibasic potassium phosphate in water/acetonitrile (9:1 v/v) as mobile phase and detection at 232 nm [107]. Lovastatin and four of its related impurities were determined on a 7 μm Nucleosil ODS column (25 cm × 4.6 mm i.d.) with borax buffer at pH 4 and methanol (3:17 v/v) as mobile phase and detection at 230 nm. The calibration graph was linear for 0.3–1.8 μg of lovastatin. The recovery was 99.8% and the RSD was 0.38%. The related impurities were separated and their contents were calculated by area normalization [108]. An Ion Pac AS 5 column, protected with an anion trap ATC-1 and a transition metal ion trap MFC-1 operated at 45 °C with a mobile phase in a gradient elution of NaOH (20–100 mM in 20 min) a self regenerating ASRS-1 suppressor and conductivity detection was studied for analysis of clodronic acid [109]. The method gave a good separation of chloride, nitrate, sulfate, phosphate clodronic acid and other organic impurities arising from the synthesis of clodronic acid. Recently, FDA has approved a synthetic pyrethroid viz., permethrin for the treatment of head lice, one of the most common diseases not only in the US but also world wide. Its use has also been extended to low mammalian toxicity and has shown to be an effective insecticide against several major insect species. Permethrin, presents two diastereomers with different chemical, physical and toxicological properties. The *cis* and *trans*-isomeric impurities and degradation products of permethrin were determined by Garcial et al. [110]. The analyses were performed on a 4 μm particle C-18 Nova-Pak (Waters) column (15 × 3.9 mm i.d.) kept in a oven at 35 °C. Mobile phase consisted of methanol–water (78:22 v/v) at a flow rate of 1 ml/min. UV detection was performed at 272 nm and peaks were identified by comparing retention times with those of standards and confirmed with characteristic spectra using the photodiode array detector. The method was used for determination of perimethrin in raw materials, pharmaceutical preparations, lotions and shampoos. It was validated following ICH recommendations. A single column ion chromatographic method with UV detection was developed for purity testing and assay of monosodium olpadronate [111]. Aqueous solution of the analyte was precipitated with methanol to enhance the impurities/olpadronate molar ratio to improve purity determination at trace levels with UV detector in indirect mode with a Waters IC Pak HR column using diluted nitric acid as the mobile phase. The method was fully validated according to ICH guidelines for the determination of phosphite, phosphate, chloride and methane sulfonic acid in olpadronate. An ion-pair LC method for determination of mangafodipir and related impurities viz., manganese (III) dipyridoxyl diphosphate sodium salt, manganese dipyridoxylmonophosphate sodium salt, manganese dipyridoxyl diphosphate monooveralkyated sodium salt and manganese (5-methyl) dipyridoxyl monophosphate sodium salt was described by Gjerde et al. [112]. Good resolution was obtained using a polymeric reverse-phase PRP-1, 5 μm column (15 cm × 4.6 mm i.d.) and a mobile phase acetonitrile–borate/terabutyl ammonium hydrogensulfate (pH 9.9; 13 mM borate, 36 mM TBA) (25:75, v/v) and the detection was at 310 nm. A large number of impurities related to 3-oxosteroids formed during the reaction steps and degradation studies were identified by Gorog et al. [113]. Their experience in the course of the separation and identification of 3-oxo related impurities in various steroid drugs such as nandrolone (19-nortestosterone) and its esters, norethisterone, norgestrel, nestorone™ (16-methylene-17a-acetoxy-19-norpregn-4-ene-3,20-dione, the experimental progestin drug of the Population Council, New York), prednisolone and mazipredone (the 21-deoxy-21-*N*-methyl-pip-erazinyl analogue of prednisolone) were summarized.

4. Conclusions

The current state-of-the art of HPLC for determination of impurities in drug substances has been reviewed. Our analysis of the published data has revealed that the HPLC is well established as an effective tool in-process control and quality assurance of bulk drugs and formulations. Of the 450 methods published during the period of 1995–2001, methods based on HPLC were 235 showing that it is the technique of choice for analysis of impurities in drugs. An average of 30 HPLC papers per year were published during the period of review. Methods based on some form of HPLC separation still out number of all other assays as they have for over two decades. In fact, this technique has set the standard against which others are compared. In principle, it is now possible to replace all non-specific assay methods adopted by USP/NF/EP/BP with highly specific and precise methods using HPLC. Currently the vast majority of drug-related impurity determinations are performed by HPLC, which offers the desired sensitivity for trace level determinations and a high degree of automation. A wide variety of stationary phases and operation modes make HPLC applicable to all drug classes. The typical detection limits for drug-related impurities by HPLC are 0.1% or lower and this can be routinely met in the majority of circumstances using conventional UV detectors. Most of the workers have used the reversed-phase mode with UV absorbance detection because this provided the best available reliability, analysis time, repeatability and sensitivity. During the period of review, HPLC was used extensively in the evaluation and control of purity of chemical components used in the manufacture of bulk drugs. A variety of approaches to establish the impurity profiles of synthetic drugs have been outlined. The bulk of these are relatively simple, isocratic reversed-phase procedures that employ UV detection. However, in some instances, more novel approaches are used that are often based on separation mode or the use of more selective detective systems typically diode array and fluorescence detectors or mass spectrometers.

In this review we have discussed the present state of the art of HPLC for determination of impurities of some important analgesic, antibiotic, anti-viral, anti-hypertensive, anti-depressant, gastro-intestinal and anti-neoplastic agents in brief. For the more common compounds such as acetaminophen, beclomethasone dipropionate, pholcodine, 4-acetylaminophenyl acetic acid, celecoxib, rocicoxib and oxaprozen a variety of analytical procedures have been developed. Many of the methods used for analysis of antibiotics continue to be based on HPLC. The most common approach used was the relatively simple isocratic reversed-phase in combination with UV detection. Methods based on gradient reversed-phase conditions are found to be very popular for analyzing the anti-viral drugs. As many as 20 methods were published, out of which most of the methods were based on gradient elution techniques for analyzing anti-hypertensive drugs. HPLC has also played a major role in the analysis of other class of compounds viz., anti-depressants, cardiovascular, steroids, gastro-intestinal etc. The particular advantage of HPLC when compared to other techniques is its chemical specificity. The HPLC approaches discussed in this review article are simple, specific and convenient.

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References

[1] J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon, A.G. Gilman (Eds.), Goodman and Gillman's The Pharmacological Basis of Therapeutics, Ninth ed., McGraw Hill, New York, 1996.
[2] S. Gorog (Ed.), Determination of Impurities in Drugs, Elsevier Sciences, Amsterdam, 1999.

[3] S. Ahuja, Impurities Evaluation of Pharmaceuticals, Marcel Dekker, New York, 1998.
[4] S. Husain, R. Nageswar Rao, Monitoring of process impurities in drugs, in: Z. Deyl, I. Miksik, F. Tagliaro, E. Tesarova (Eds.), Advanced Chromatographic and Electromigration Methods in Biosciences, Elsevier Sciences, Amsterdam, 1998, pp. 834–888.
[5] K.D. Altria, J. Chromatogr. 735 (1996) 43–56.
[6] E.M. Sheldon, J.B. Downar, J. Pharm. Biomed. Anal. 20 (2000) 561–572.
[7] G.J. Lehr, T.L. Barry, G. Petzinger, G.M. Hanna, S.W. Zito, J. Pharm. Biomed. Anal 19 (1999) 373–389.
[8] D.D. Wirth, B.A. Olsen, D.K. Hallenbeck, M.E. Lake, S.M. Gregg, F.M. Perry, Chromatographia 46 (1997) 511–523.
[9] International Conference on the Harmonization of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Q3A: Impurities in New Drug Substances, January 1996.
[10] P. Zanowiak, in: Kirk-Othmer (Ed.), Encylopedia of Chemical Technology, vol. 18, Fourth ed., Wiley, Chichester, 1996, pp. 480–510.
[11] ANDAs: Impurities in drug products, Guidance for Industry, US Food and Drug Administration, Center for Drug Evaluation and Research, Department of Health and Human Services, 1998.
[12] ICH. Q3A: Impurities in New Drug Substances (January 1996) and Q3B: Impurities in New Drug Products, May 1997.
[13] The United States Pharmacopoeia 24, USP Convention Inc., Rockville, 2000, p. 1876 and p. 2049.
[14] K.K. Maggon, A. Mechkovsi, Drug News Perspect. 5 (1992) 261–270.
[15] F. Erni, P. Helboe, Pharma times 10 (1996) 9–15.
[16] Guidelines for Submitting Samples and Analytical Data for Methods Validation, US Food and Drug Administration, Center for Drugs and Biologics, Department of Health and Human Services, 1987.
[17] CDER Guideline on Validation of Chromatographic Methods, Reviewer Guidance of Chromatographic Methods, US Food and Drug Administration, Center for Drugs and Biologics, Department of Health and Human Services, 1994.
[18] ICH, Q2A: Validation of Analytical Methods, Definitions and Terminology, October 1994.
[19] ICH, Q2B: Analytical Validation-Methodology, November 1996.
[20] D. Saletan, Creative Troubleshooting in the Chemical Process Industries, Blackie Academic Professional, New York, 1996, pp. 284–300.
[21] The United States Pharmacopoeia XV, 15th revision, Mack Publishing Company, Easton, 1955, p. 168 and p. 187.
[22] S. Gorog, J. Brlik, A. Csehi, Zs. Halmos, B. Herenyi, P. Horvath, F. Dravecz, D. Bor, Anal. Method. Instrum. 2 (1995) 154–157.
[23] P. Cunnif (Ed.), Official Methods of Analysis of AOAC International, 16th ed., vol. 1. AOAC International, Arlington, 1995.
[24] R.K. Gilpin, Pharmaceutical and drugs, in: R.A. Meyers (Ed.), Encyclopedia of Analytical Chemistry, vol. 8, Wiley, Chichester, 2000.
[25] G. Szepesi, S. Nyiredy, Pharmaceuticals and drugs, in: J. Sherma, B. Fried (Eds.), Hand Book of Thin-layer Chromatography, Second ed., Mercel Dekker, New York, 1996, pp. 819–876.
[26] D.G. Watson, Pharmaceutical Analysis, Churchill Livingstone, Edinburg, 1999, pp. 208–235.
[27] M.H. Lee, K.E Markides, Analytical Supercritical Fluid Chromatography and Extraction, Chromatography Conferences, Utah, 1990.
[28] H. Shintani, J. Polonsky (Eds.), Hand Book of Capillary Electrophoresis Applications, Blakie Academic and Professional, London, 1997.
[29] H. Nishi, Electrophoresis 20 (1999) 3237–3258.
[30] R.K. Gilpin, L.A. Pachla, Anal. Chem. 73 (2001) 2805–2816.
[31] A.M. Krstulovic, C.R. Lee, J. Chromatogr. B. Biomed. Appl. 689 (1997) 137–153.
[32] P.D. Sethi, High Performance Liquid Chromatography, CBS Publishers, New Delhi, 2001.
[33] P.C. Sadek, Troubleshooting HPLC Systems—A Bench Manual, Wiley, New York, 2000.
[34] T. Arai, J. Chromatogr. B. Biomed. Appl. 717 (1998) 295–311.
[35] E. Tesarova, D.W. Armstrong, J. Chromatogr. Libr. 60 (1998) 197–256.
[36] O.I. Kalchenko, V.A. Golub, I.V. Zavatskaja, J. Pharm. Biomed. Anal. 14 (1995) 107–111.
[37] W. Naidong, S. Hua, E. Roets, J. Hoogmartens, J. Pharm. Biomed. Anal. 13 (1995) 905–910.
[38] C.E. Thomson, M.R. Gray, M.P. Baxter, J. Pharm. Biomed. Anal. 15 (1997) 1103–1111.
[39] B.A. Olsen, S.W. Baertschi, R.M. Riggin, J. Chromatogr. 648 (1993) 165–173.
[40] J. Ermer, M. Vogel, Biomed. Chromatogr. 14 (2000) 373–383.
[41] C.J. Govaerts, J. Orwa, A. van Schepdael, E. Roets, J. Hoogmartens, Rapid Commun. Mass Spectrom. 15 (2001) 128–134.
[42] W.M.A. Niessen, J. Chromatogr. A 856 (1999) 179–197.
[43] J. Ermer, J. Pharm. Biomed. Appl. 18 (1998) 707.
[44] E.C. Nicolas, T.H. Scholz, J. Pharm. Biomed. Appl. 16 (1998) 825.
[45] R.K. Gilpin, L.A. Pachla, Anal. Chem. 71 (1999) 217R–233R.
[46] S. Husain, R.N. Nageswara Rao, Proc. Control Qual. 10 (1997) 41–57.
[47] S. Gorog, M. Babjak, G. Balogh, J. Brlik, A. Csehi, F. Dravecz, M. Gazdag, P. Horvath, A. Lauko, K. Varga, Talanta 44 (1997) 1517–1526.
[48] E.R. Montgomery, A.L. Edmanson, S.C. Cook, P.K. Hovsepian, J. Pharm. Biomed. Anal. 25 (2001) 267–284.

[49] B.L. Hogan, M. Williams, A. Idiculla, T. Veysoglu, E. Parente, J. Pharm. Biomed. Anal. 23 (2000) 637–651.
[50] C. Eckers, N. Haskins, J. Langridge, Rapid Commun. Mass Spectorm. 11 (1997) 1916–1922.
[51] C. Govaerts, H.K. Chepkwony, A. vanSchepdael, E. Roets, J. Hoogmartens, Rapid Commun. Mass Spectrom. 14 (2000) 878–884.
[52] S. Talor, S. Preece, Micromass Application Note, 227 (1997) 5.
[53] L.A. Shervington, N. Sakhnini, J. Pharm. Biomed. Anal. 24 (2000) 43–49.
[54] D. De Orsi, L. Gagliardi, F. Chimenti, D. Tonelli, Anal. Lett. 28 (1995) 1655–1663.
[55] O.M. Denk, D.G. Watson, G.G. Skellern, J. Chromatogr. A 895 (2000) 9–16.
[56] J.H.Mc.B. Miller, P. Ceva, V. Dujardin, G.G. Skellern, S. Herne, Pharmeuropa 10 (1998) 502–503.
[57] F.E. Blondino, P.R. Byron, J. Pharm. Biomed. Anal. 13 (1995) 111–119.
[58] J. Fu, Y.Q. Zhang, D.Y. Xu, Yaowu Fenxi Zazhi 19 (1999) 345–347.
[59] M.K. Sreenivasu, Ch. Lakshmi Narayana, D. Sreenivasa Rao, G. Om Reddy, J. Pharm. Biomed. Anal. 22 (2000) 949–956.
[60] T. Radhakrishna, D.S. Rao, G. Om Reddy, J. Pharm. Biomed. Anal. 26 (2001) 617–628.
[61] K.V.S.K. Reddy, D.S. Rao, K. Vyas, G. Om Reddy, J. Pharm. Biomed. Anal. 22 (2000) 651–659.
[62] F. Fang, G.J. Xu, B. Lu, J. Liq. Chromatogr. Relat. Technol. 24 (2001) 1021–1027.
[63] S. Hess, M. Akermann, D. Ropte, K. Eger, J. Pharm. Biomed. Anal. 25 (2001) 531–538.
[64] W.J. Welsh, W. Lin, S.H. Terrigni, E. Collantes, R. Duta, M.S. Carey, W.L. Zieliski, J. Brower, J.A. Spencer, T.P. Layloff, Anal. Chem. 68 (1996) 3473–3482.
[65] J. Ito, Y. Hosaki, Y. Torigoe, K. Sakimoto, Food Chem. Toxicol. 1 (1992) 71–81.
[66] European Pharmacopoeia, Supplement, third ed., Council of Europe, Strasbourg, 2000, p. 1280.
[67] A. Ekpe, J.H. Tong, L. Rodriguez, J. Chromatogr. Sci. 39 (2001) 81–86.
[68] J.A. Orwa, K. Vandenbempt, S. Depuydt, E. Roets, J. Hoogmartens, J. Pharm. Biomed. Anal. 20 (1999) 745–752.
[69] J.H.Mc.B. Miller, Pharmeuropa 12 (2000) 8–17.
[70] H. Russ, D. Mc Cleary, R. Katimy, J.L. Montana, R.B. Miller, R. Krishnamoorthy, C.W. Davies, J. Liq. Chromatogr. Relat. Technol. 21 (1998) 2165–2181.
[71] W.X. Zhang, C.G. Jiang, G.L. Guo, Yaowu Fenxi Zazhi 18 (1998) 314–316.
[72] J.A. Orwa, F. Bosmans, S. Depuydt, E. Roets, J. Hoogmartens, J. Chromatogr. A 829 (1998) 161–166.
[73] O. Shakoor, R.B. Taylor, Analyst 121 (1996) 1473–1478.
[74] Y. Okamoto, K. Itoh, Y. Namiki, J. Matsuhita, M. Fujisoka, T. Yasuda, J. Pharm. Biomed. Anal. 14 (1996) 739–748.
[75] S. Husain, S. Khalid, V. Nagaraju, R. Nageswara Rao, J. Chromatogr. 705 (1995) 380–384.
[76] R. Vogel, K. DeFillipo, V. Reif, J. Pharm. Biomed. Anal. 24 (2001) 405–412.
[77] M.M. Caamano, L.V. Garcia, B. Elorza, J.R. Chantres, J. Pharm. Biomed. Anal. 21 (1999) 619–624.
[78] C.Q. Li, T. Tougas, K. Cohen, R. Lee, P. Meagan, M. Corson, T. Muchnik, J. Chromatogr. Sci. 38 (2000) 246–254.
[79] V.V. Vaidya, M. Khanolkar, J.N. Gadre, Indian Drugs 37 (2000) 521–523.
[80] A.R. Gomes, V. Nagaraju, J. Pharm. Biomed. Anal. 26 (2001) 919–927.
[81] V. Marinkovic, D. Agbaba, S. Vladimirov, S. Stankovic, J. Pharm. Biomed. Anal. 24 (2001) 993–998.
[82] L. Valvo, R. Alimenti, S. Alimonti, S. Raimondi, F. Foglietta, F. Campana, J. Pharm. Biomed. Anal. 17 (1997) 989–996.
[83] T. Radhakrishna, D.S. Rao, K. Vyas, G.Om. Reddy, J. Pharm. Biomed. Anal. 22 (2000) 641–650.
[84] D. De Orsi, L. Gagliardi, L. Turchetto, D. Tonelli, J. Pharm. Biomed. Anal. 17 (1998) 891–895.
[85] G.Z. Yin, Sepu 14 (1996) 308–309.
[86] J.S. Munro, T.A. Walker, J. Chromatogr. A 914 (2001) 13–21.
[87] Z. Halmos, C. Szantay, Jr., J. Brlik, A. Cschi, K. Varga, P. Horvath, M. Kislaki, G. Domany, A. Nemes, S. Gorog, J. Pharm. Biomed. Anal. 15 (1996) 1–5.
[88] S. Husain, S. Khalid, V. Nagaraju, R. Nageswara Rao, J. Chromatogr. A 743 (1996) 328–334.
[89] R. Lipka, M. Sobezak, S. Kus, S. Orzwaldowski, M. Jarosz, Microchem. J. 65 (2000) 51–58.
[90] I. Cendrowska, B. Buszewski, J. Liq. Chromatogr. Relat. Technol. 22 (1999) 2259–2270.
[91] J. Chen, Z.Y. Huang, Sepu 17 (1999) 85–86.
[92] K. Brightman, G. Finlay, I. Jasvis, T. Knowlton, C.T. Manktelow, J. Pharm. Biomed. Anal. 20 (1999) 439–447.
[93] F.Y. Pan, H.D. Liang, Fenxi Huaxue 26 (1998) 528–530.
[94] L.K. Sho, D.C. Locke, Anal. Chem. 69 (1997) 2008–2016.
[95] S. Gliesing, D. De La Calle, M. Reichenbaecher, M. Boese, Pharmazie 52 (1997) 399–407.
[96] H.Y. Aboul-Enein, V. Serignese, Anal. Chim. Acta 319 (1996) 187–190.
[97] D.S. Rao, S. Geetha, M.K. Srinivasu, G. Om Reddy, J. Pharm. Biomed. Anal. 26 (2001) 629–636.
[98] S.T. Eolgan, M.L. Dumont, S.G. Ruggeri, J. Pharm. Biomed. Anal. 18 (1998) 429–440.
[99] F. Belal, H.A. Al-Malaq, A.A. Al-majed, E.A. Gadkariem, J. Liq. Chromatogr. Relat. Technol. 23 (2000) 3175–3189.
[100] D. Orlovic, D.M. Radulovic, D. Ivanovic, Z.B. Vujic, Chromatographia 52 (2000) 732–734.
[101] P.M. Lacroix, P.N. Yat, E.G. Loviring, J. AOAC. Int. 78 (1995) 334–339.
[102] X. Xu, J.T. Stewart, J. Liq. Chromatogr. Relat. Technol. 22 (1999) 1857–1866.

[103] A.P. Micheel, C.Y. Ko, H.Y. Gun, J. Chromatogr. B. Biomed. Appl. 709 (1998) 166–172.
[104] R. Mansfield, J. Huang, S. Thatcher, R.B. Miller, C.W. Davis, J. Liq. Chromatogr. Relat. Technol. 22 (1999) 2187–2209.
[105] P. Coufal, K. Stulik, H.A. Claessens, M.J. Hardy, M. Webb, J. Chromatogr. B. Biomed. Appl. 732 (1999) 437–444.
[106] M. Rajevic, P. Betto, J. Liq. Chromatogr. Relat. Technol. 21 (1998) 2821–2830.
[107] E.M. Chellquist, W.O. Nelson, H.L. Storflor, J. Pharm. Biomed. Anal. 16 (1997) 39–45.
[108] Z.F. Zhang, Y. Lu, Y.D. Wang, G.J. Liang, Yaowu Fenxi Zazhi 16 (1996) 373–376.
[109] G.E. Taylor, J. Chromatogr. A 770 (1997) 261–271.
[110] E. Garcial, A. Garcia, C. Barbas, J. Pharm. Biomed. Anal 24 (2001) 999–1004.
[111] O. Quattrocchi, L. Frisardi, M. Iglesias, M. Noya, M. Caputto, D. Ferraris, D. Siliprandi, E. Piccinni, J. Pharm. Biomed. Anal. 24 (2001) 1011–1018.
[112] H. Gjerde, A. Snotun, H. Hem, K.H. Larsen, J. Dohl, J. Pharm. Biomed. Anal 25 (2001) 109–114.
[113] S. Gorog, M. Babjak, G. Balogh, J. Brlik, F. Dravecz, M. Gazdag, P. Horvath, A. Lauko, K. Varga, J. Pharm. Biomed. Anal. 18 (1998) 511–525.
[114] I. Medina, Analusis 27 (1999) 447–451.
[115] D. Song, E. Roets, J. Hoogmartens, Pharmeuropa 11 (1999) 432–436.
[116] B. Di, F. Fang, Y.Y. Li, L.J. Yu, H.L. Song, Z.X. Zang, Yaowu Fenxi Zazhi 19 (1999) 293–296.
[117] V. Harang, D. Westerlund, Chromatographia 50 (1999) 525–531.
[118] K.R.P. Shenoy, G.N. Prashant, Indian Drugs 36 (1999) 632–634.
[119] J.H.Mc.B. Miller, H. Binder, R. de Richter, G. Decristoforo, A. Mayrhofer, E. Roets, S. Saarlo, C. Vander Vlies, Pharmeuropa 12 (2000) 3–7.
[120] J.J.N. Cirunay, Y. Vander Heyden, J. Plaizer-Vercammen, J. Chromatogr. Sci. 36 (1998) 417–421.
[121] J.N.A. Tettey, G.G. Skellern, J.M. Midgley, M.H. Grant, J. Pharm. Biomed. Anal. 17 (1998) 713–718.
[122] T.W. Ryan, Anal. Lett. 31 (1998) 2447–2456.
[123] P.M. Lacroix, B.A. Dawson, R.W. Sears, D.B. Black, T.D. Cyr, J.C. Ethier, J. Pharm. Biomed. Anal. 18 (1998) 383–402.
[124] J.H.Mc.B. Miller, P. Ceva, V. Dujardin, G.G. Skellern, S. Herne, Pharmeuropa 10 (1998) 502–503.
[125] N. Cillat, G. Decristoforo, P. Inama, R. Macas, A. Mayrhofer, J.H.Mc.B. Miller, C. Vander Vlies, M. Wieser, Pharmeuropa 10 (1998) 510–518.
[126] S. Rubnov, D. Levy, H. Schneider, J. Pharm. Biomed. Anal. 18 (1999) 939–945.
[127] A.L.S. Pena, A. Carmona, A. Barbora, C.M. Lino, I.N. Silveira, B. Castillo, J. Pharm. Biomed. Anal. 18 (1998) 839–845.
[128] B.A. Avery, K.K. Venkatesh, M.A. Avery, J. Chromatogr. B. Biomed. Appl. 730 (1999) 71–80.
[129] S.C. Pefile, E.W. Smith, P.B. Kruger, C.F. Albrect, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 3061–3072.
[130] M. Zhou, Y.S. Huang, Y.Q. Sun, Yaowu Fenxi Zazhi 17 (1997) 366–368.
[131] H. Yang, F.C. Thyrion, J. Liq. Chromatogr. Relat. Technol. 21 (1998) 1347–1357.
[132] J.H.Mc.B. Miller, T. Burat, C.P. Christiansen, N. Cillat, J. Hoogmartens, P. Inama, R.K. De Richter, A. Mayrhofer, E. Roets, K. Sinivuo, Pharmeuropa 10 (1998) 149–153.
[133] W. Jiang, R. Ji, Supelco Rev. 17 (1998) 9.
[134] I.E. Panderi, H.A. Archontaki, E.E. Gikas, M. Parisi-Poulou, J. Liq. Chromatogr. Relat. Technol. 21 (1998) 1783–1795.
[135] C.X. Wang, G.W. Zou, Sepu 16 (1998) 255–257.
[136] I. Panderi, H. Archnotaki, E. Gikar, M. Parissi-Poulou, J. Pharm. Biomed. Anal. 17 (1998) 327–335.
[137] R. Gatti, R. Gotti, M.G. Gioia, V. Cavrini, J. Pharm. Biomed. Anal. 17 (1998) 337–347.
[138] A.L. Cornaz Gudet, P. Buri, STP Pharma Sci. 8 (1998) 139–144.
[139] A.I. Segall, M.F. Vitale, V.L. Perz, M.L. Palacios, M.T. Pizzorno, J. Liq. Chromatogr. Relat. Technol. 23 (2000) 1377–1386.
[140] A.P. Argekar, S. Shah, Indian Drugs 33 (1996) 453–457.
[141] J.H.Mc.B. Miller, A. Aranda, T. Burat, K. Fischer, A. Klemann, M.G. Kister, A. Mayrhofer, Pharmeuropa 8 (1996) 434–442.
[142] J.H.Mc.B. Miller, J. De Beer, V. Hartofylax, J. Hoogmartens, M.G. Kister, B. Serckx, Y. Zhy, Pharmeuropa 8 (1996) 443–450.
[143] T. Simat, B. van Wickern, K. Fulitz, H. Steinhart, J. Chromatogr. B. Biomed. Appl. 685 (1996) 41–51.
[144] J.T. Walker, D.L. Paolini, J. Segretario, J. Chromatogr. Sci. 34 (1996) 513–516.
[145] A.I. Chernyshev, S.V. Kuz Min, V.P. Zhestkov, L.D. Smirnov, Khim-Farm. Zh. 5 (1996) 63–64.
[146] G.J. Mac Eachem-Keith, L.J. Wagner Butterfield, M.J.I. Mattina, Anal. Chem. 69 (1997) 72–77.
[147] R. Ferretti, B. Gallinella, F. La Torre, L. Turchetto, J. Chromatogr. A 769 (1997) 231–238.
[148] T.M. Wilox, S.R. Hagen, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 1539–1551.
[149] S.V. Kuzmin, E.E. Ishina, M.L. Shishova, V.A. Kozlov, Khim-Farm. Zh. 31 (1997) 55–56.
[150] J.P. Basly, J.L. Duroux, M. Bernard, J. Pharm. Biomed. Anal. 15 (1997) 1137–1141.
[151] S.L. Hoerle, B.G. Snider, J. Liq. Chromatogr. 18 (1995) 3269–3289.
[152] D.S. Risely, J.A. Peterson, J. Liq. Chromatogr. 18 (1995) 3035–3048.
[153] J.N. Yu, S.T. Tian, K.Y. Ni, G.X. Cheng, Z.G. Li, Yaowu Fenxi Zazhi 15 (1995) 28–32.
[154] Z.F. Zhang, Y. Zhou, S.H. Jin, Yaowu Fenxi Zazhi 15 (1995) 17–19.
[155] K. Sustacha, M. Chacon, M.L. Lucero, J. Chromatogr. 719 (1996) 245–250.

[156] W.H. Doub, D.D. Ruhl, B. Hart, P.R. Mehelic, L.K. Revelle, J. AOAC. Int. 79 (1996) 636–639.
[157] A.M. Al-Obaid, M.E.M. Hagga, I.E. El-Khawad, O.H.M. El-Mahi, J. Liq. Chromatogr. Relat. Technol. 19 (1996) 1369–1389.
[158] G.M. Duignan, J.H.Mc.B. Miller, B. Miller, G.B. Skellern, J. Pharm. Biomed. Anal. 14 (1996) 451–456.
[159] R. Ferretti, B. Gallinella, F. La Torre, A. Lusi, J. Chromatogr. A 731 (1996) 340–345.
[160] G.M. Zeng, D.H. Yu, S.M. Qui, X.H. Guo, M. Sakai, Z.L. Li, Fenxi Kexue Xuebao 12 (1996) 23–26.
[161] S.T. Colgan, G.R. Haggan, R.H. Reed, J. Pharm. Biomed. Anal. 14 (1996) 825–833.
[162] J.H.Mc.B. Miller, C.J. Nap, Pharmeuropa 8 (1996) 179–186.
[163] Y. Zhu, E. Roets, M.L. Morene, E. Porquers, J. Hoogmartens, J. Liq. Chromatogr. Relat. Technol. 19 (1996) 1893–1908.
[164] D. Simpson, G.G. Skillern, J.H.Mc.B. Miller, J. Pharm. Biomed. Anal. 14 (1996) 1371–1375.
[165] E.M. Abdel-Moety, K.A. Rashood, A. Rauf, N.A. Khattab, J. Pharm. Biomed. Anal. 14 (1996) 1639–1644.
[166] A.P. Argekar, S.U. Kapadia, S.V. Raj, S.S. Kunjir, Indian Drugs 33 (1996) 261–266.
[167] N. Beaulieu, R.W. Sears, E.G. Lovering, J. AOAC Int. 77 (1994) 857–861.
[168] K.M. Valentik, E. Karaffa, L. Ladanyi, Acta Pharm. Hung. 63 (1993) 57–65.
[169] V. Ulvi, H. Keski-Hynnila, Am. Lab. 26 (1994) 56–59.
[170] D.A. Barrett, P.N. Shaw, J. Liq. Chromatogr. 17 (1994) 3727–3733.
[171] D. Conklin, T. Ascah, Supelco Rep. 13 (1994) 5–6.
[172] J. Waitita Muritu, I. Congubokibwage, C. Karimi Maitai, J. Hoogmartens, J. Pharm. Biomed. Anal. 12 (1994) 1483–1488.
[173] Y.X. Zhu, C. Hendrix, R. Busson, G. Janssen, E. Roets, J. Hoogmartens, J. Pharm. Biomed. Anal. 12 (1994) 1137–1140.
[174] P.M. Lacroix, N.M. Curran, W.H. Sy, D.K.J. Gorecki, P. Thibault, P.K.S. Blay, J. AOAC Int. 77 (1994) 1447–1453.
[175] D. Agbaba, V. Janjic, D. Zivanov-Stakic, S. Vladimirirov, J. Liq. Chromatogr. 17 (1994) 3983–3988.
[176] I. Sakano, T. Ishii, S. Ichikawa, K. Harasawa, K. Minohara, S. Yamamura, S. Nishiyama, J. Pharm. Sci. 83 (1994) 1363–1366.
[177] B.S. Lord, R.W. Stringham, Anal. Chem. 66 (1994) 4370–4374.
[178] L.X. Ding, K.Y. Ni, Y.Y. Chen, Y.Z. Hu, S.L. Qiu, R.G. Yu, Yaowu Fenxi Zazhi 15 (1995) 3–6.
[179] G.L. Hoyer, J. Le Doux, P.E. Nolan, Jr., J. Liq. Chromatogr. 18 (1995) 1239–1249.
[180] N. Beaulieu, P.M. Lacroix, R.W. Sears, E.G. Lovering, J. AOAC. Int. 78 (1995) 647–650.
[181] K. Torniainen, E. Maki, J. Chromatogr. 697 (1995) 397–405.
[182] J. Paesen, P. Claeyr, W. Cypers, E. Roets, J. Hoogmartens, J. Chromatogr. A 699 (1995) 93–97.
[183] R. Ferretti, B. Gallinella, F. La Torre, J. Chromatogr. A 704 (1995) 217–223.
[184] N. Daraghmeh, M. Al-Omari, A.A. Badwan, A.M.Y. Jaber, J. Pharm. Biomed. Anal. 25 (2001) 483–492.
[185] A.P. Cheung, E. Struble, N. Nguyen, P. Liu, J. Pharm. Biomed. Anal. 24 (2001) 957–966.
[186] S. Hou, M. Hindle, P.R. Byron, J. Pharm. Biomed. Anal. 24 (2001) 371–380.
[187] Z.H. Gao, D.N. Luo, Yaowu Fenxi Zazhi 21 (2001) 163–164.
[188] M. Bakshi, B. Singh, A. Singh, S. Singh, J. Pharm. Biomed. Anal. 26 (2001) 891–897.
[189] J. Lambropoulos, G.A. Spanos, N.V. Lazaridis, T.S. Ingallinera, V.K. Rodriguez, J. Pharm. Biomed. Anal. 20 (1999) 705–716.
[190] A.S. Low, J. Wangboonskul, Analyst 124 (1999) 1589–1593.
[191] V. Andrisano, R. Gotti, A. Leoni, V. Cavrini, J. Pharm. Biomed. Anal. 21 (1999) 851–857.
[192] F. Hudrea, C. Grosset, J. Alary, M. Bojita, Biomed. Chromatogr. 14 (2000) 17–18.
[193] P.J. Simmr, R.W. Towne, C.S. Gross, R.E. Miller, J. Pharm. Biomed. Anal. 17 (1998) 841–849.
[194] Q.M. Guan, Z.X. Li, Q.L. Zhang, Yaowu Fenxi Zazhi 18 (1998) 300–302.
[195] M.A. Quarry, R.C. Williams, D.S. Sebastian, J. Liq. Chromatogr. Relat. Technol. 21 (1998) 2841–2852.
[196] S.A. Benetton, E.R.M. Kedor-Hackmann, M.I.R.M. Santoro, V.M. Borges, J. Liq. Chromatogr. Relat. Technol. 21 (1998) 3215–3221.
[197] M. Jimidar, N. Niemeijer, R. Peeters, J. Hoogmartens, J. Pharm. Biomed. Anal. 18 (1998) 479–485.
[198] G.H. Cheng, Yaowu Fenxi Zazhi 19 (1999) 274–275.
[199] J. Palsen, E. Roets, J. Hoogmartens, J. Pharm. Biomed. Anal. 17 (1998) 53–56.
[200] T.Y. Fan, G.M. Wall, K. Sternitzke, L. Bass, M.B. Morton, E. Muegge, J. Chromatogr. A 740 (1996) 289–295.
[201] L. Valve, S. Alimonti, R. Alimenti, C. De Sona, E. Ciranni Signoretti, R. Draisci, L. Giannetti, J. Pharm. Biomed. Appl. 15 (1997) 487–493.
[202] M.M. Nasr, T.J. Tschappler, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 553–565.
[203] G. Wang, R.B. Miller, L. Melendez, R. Jacobus, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 567–581.
[204] A.K. Handa, V.P. Shedbalkar, H.L. Bhalla, Indian Drugs 33 (1996) 559–562.
[205] J. Zhang, R.B. Miller, R. Jacobus, Chromatographia 44 (1997) 247–252.
[206] A.H. Schmidt, K.-O. Hiller, GIT Fachz Lab. 40 (1996) 1100–1102.
[207] E. Bousquet, N.A. Santagati, S. Tirendi, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 757–770.
[208] P.W. Haney, A.K. Dash, J. Chromatogr. A 765 (1997) 233–239.

[209] A.R. Barnes, J. Liq. Chromatogr. 18 (1995) 3117–3128.
[210] E. Kourany-Lefoll, T.D. Cyr, Can. J. Appl. Spectorosc. 40 (1995) 155–159.
[211] Y. Ha, A.P. Cheung, J. Pharm, Biomed. Anal. 14 (1996) 1327–1334.
[212] J.P. Bounine, B. Tardif, P. Beltran, D.J. Mazzo, J. Chromatogr. A 677 (1994) 87–93.
[213] Y. Shah, S. Joshi, K.C. Jindal, S. Khanna, Drug Dev. Ind. Pharm. 20 (1994) 85–91.
[214] X. Wang, Yaowu Fenxi Zazhi 15 (1995) 23–26.
[215] F. Belal, I.A. Al-Zaagi, E.A. Gadkariem, M.A. Abounassif, J. Pharm. Biomed. Anal. 24 (2001) 335–342.
[216] X.J. Guo, Z.Y. Wei, F.M. Li, Sepu 19 (2001) 279–280.