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Review

Determination of paracetamol: Historical evolution

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Abstract

Paracetamol is a common analgesic and antipyretic drug that is used for the relief of fever, headaches and other minor aches and pains. Their determination in pharmaceuticals is of paramount importance, since an overdose of paracetamol can cause fulminating hepatic necrosis and other toxic effects. Many analytical methodologies have been proposed for the determination of paracetamol. The aim of the present study is to evaluate the utility of different techniques for quantification of paracetamol content in pharmaceutical formulations and biological samples.

Keywords: Paracetamol; Review; Pharmaceutical formulations; Biological fluids

Contents

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1. Introduction

Paracetamol (PCT) is an acylated aromatic amide, which was firstly introduced into medicine as an antipyretic/analgesic by Von Mering in 1893 and has been in use as an analgesic for home medication for over 30 years and is accepted as a very effective treatment for the relief of pain and fever in adults and children. It is the most used medicine after acetylsalicylic acid in many countries as an alternative to aspirin and phenacetin. PCT is also known as acetaminophen (*N*-acetyl-*p*-aminophenol, 4-acetamidophenol); it is a major ingredient in numerous cold and flu medications and many prescription analgesics. It is remarkably safe in standard doses, but, because of its wide availability, deliberate or accidental overdoses are not uncommon. PCT, unlike other common analgesics such as aspirin and ibuprofen, has no anti-inflammatory properties, and so it is not a member of the class of drugs known as *non-steroidal anti-inflammatory drugs* or NSAIDs. In normal doses, PCT does not irritate the lining of the stomach or affect blood coagulation, the kidneys, or the fetal ductus arteriosus. Like NSAIDs and unlike opioid analgesics, PCT has not been found to cause euphoria or alter mood in any way. PCT and NSAIDs have the benefit of bearing no risk of addiction, dependence, tolerance and withdrawal. Data about chemical, physical and biopharmaceutical properties are easily available.

Recent studies have shown that PCT is associated to hepatic toxicity and renal failure despite of its apparent innocuous character. Hepatic toxicity begins with plasma levels of PCT in the 120 $\mu g\,ml^{-1}$ range 4 h after the ingestion and an acute damage is presented with plasmatic levels up to 200 $\mu g\,ml^{-1}$ 4 h after the ingestion.

At normal therapeutic doses, PCT is metabolised very fast and completely by undergoing glucuronidation and sulphation to inactive metabolites that are eliminated in the urine. However, PCT higher doses produce toxic metabolite accumulation that causes hepatocyte death. Acetaminophen overdose is a frequent cause of fulminating hepatic failure in Europe and US. Based on the aforementioned observations the main objective was to carry out the development of more efficient analytical techniques, destined to quality control of one of the medicaments more widely used. Several methods have been utilised for its determination in pure form, formulation and combination with other substances; mainly volumetric, polarographic, UV–vis spectrophotometric, fluorimetric, chromatographic and many others.

The aim objective of this review is classified, summarised and discusses the different proposed methods for the determination of PCT, alone and in mixtures, in formulations and biological samples. The determinations are classified into five main categories: (1) optical methods; (2) electroanalytical methods; (3) chromatographic methods; (4) capillary electrophoretic methods; (5) water analysis.

2. Optical methods

2.1. Determinations in formulations

2.1.1. UV/vis spectrophotometric methods

PCT has been determined by titrimetric procedures for many years [1–19]. Murfin et al. [20–24] developed a continuous, auto-analytical system for the analysis by a colorimetric method of pharmaceutical formulations containing phenacetin (*N*-acetyl-9-phenetidine) or PCT (9-acetamidophenol). A method based on measuring the intensity of the yellow colour that developed when acute PCT is allowed to react with *p*-dimethylaminobenzaldehyde in 2 M HCl after heating are described [25]. Also, PCT was determined by hydrolysis with H_2SO_4, diazotisation of the *p*-aminophenol obtained followed by coupling with β-naphthol in alkaline medium [26]. Most of the published colorimetric methods for the determination of phenacetin and paracetamol require their initial de-acetylation by boiling with a mineral acid for up to 2 h, followed by application of a standard procedure to the resulting primary aromatic amine, *p*-phenetidine (9-aminoethoxybenzene) or 9-aminophenol. An automated method [27] has been described for the determination of PCT by hydrolysis, diazotisation of the amine produced and coupling the resulting product with 1-naphthol, but this process, which includes the need to heat and cool the solution, is rather lengthy. Two colorimetric manual methods have been suggested that do not require initial hydrolysis of the phenacetin and paracetamol; one involves heating them with 10% nitric acid [28] and the other warming with a solution of chloramine T [29]. Neither method proved sensitive enough for the desired automatic system.

Automatic [20] and manual [21] colorimetric procedures for the determination of paracetamol and phenacetin based on the indophenol reaction were described. The compound to be determined is made to react with acidified hypochlorite solution to form a quinonechlorimide, excess of hypochlorite is reduced with As(III) and the quinonechlorimide is made to react with phenol to form an indophenol dye. An alternative manual procedure is described for the colorimetric determination of phenacetin and paracetamol as indophenol dyes [22]. The procedure differs from that described in [21] in that phenacetin and paracetamol are hydrolysed first to *p*-phenetidine and *p*-aminophenol, respectively, before reaction with hypochlorite to form *p*-quinonechlorimide, which then undergoes a reaction with phenol.

The spontaneous oxidation of alkaline mixtures of *p*-aminophenol and phenol with molecular oxygen to form indophenol has been made the basis of a colorimetric procedure for the determination of PCT via its hydrolysis product, 9-aminophenol [30]. Total paracetamol and phenacetin can be determined by an indophenol reaction after hydrolysis and oxidation with acidified hypochlorite. Other simple and sensitive spectrophotometric method for the assay of three antipyretic drugs through their nitration and subsequent complexation with a nucleophilic reagent is proposed by El Kheir et al. [31]. Also,

dimedone was used as an analytical reagent for the colorimetric determination of PCT and oxyphenbutazone [32].

When there are no significant spectral interferences, the determination of PCT in pharmaceutical products can be carried out by direct UV absorption spectrophotometry, such as in the Paracetamol Tablets monograph in the *British Pharmacopoeia* 1999 [33]. However, when formulated with other UV absorbing substances such as excipients or active substances, where spectral overlap is possible, separative techniques such as high performance liquid chromatography (HPLC) are usually necessary, as prescribed, for instance, in several of the acetaminophen articles in the USP 24 [34]. The determination of PCT has been reported employing flow-injection systems (FIA) [35–37] with colorimetric detection. Usually, these methods are based on the hydrolysis of paracetamol to 4-aminophenol, which is then transformed into a coloured compound by a suitable reaction [38–40]. This strategy, however, has the disadvantage that the hydrolysis step requires high temperatures, which are usually attained by placing the flow reactor in a heating bath or inside a microwave oven. The hot flow coming out from the reactor should then be cooled to room temperature by means of an ice bath or similar cooling device, which adds up to the final complexity of the system. Thus, a reaction that could be carried out at room temperature would lead to a simpler system and should be preferred. PCT reacts with nitrous acid at room temperature under mild conditions (10%, m/v, $NaNO_2$, 50%, v/v, HCl) producing a derivative whose absorbance can be measured in alkaline solution at 430 nm. This reaction was investigated by Le Perdriel et al. [41] and by Chafetz et al. [42]. Other FI method is based on the nitration of PCT with sodium nitrite, and the absorption of the reaction product is measured at 430 nm in alkaline medium [43].

Direct UV/vis spectrometry is by far the instrumental technique of choice in industrial laboratories, owing mainly to its simplicity, often demanding low cost equipment. The majority published spectrophotometric methods are based on indophenol dye, and Schiff's base formation, nitrosation and subsequent chelation, oxidation, oxidative coupling with some special reagent, UV absorption, pH-induced spectral changes [44–86]. Various colour reactions have been used involving hydrolysis to 4-aminophenol and formation of a Schiff base with a substituted benzaldehyde [45–49] or diazotisation and coupling [49]. Other methods are based on indophenol formation [28,30,50], nitrosation and subsequent chelation [51], UV absorption [52] and its change with pH [53]. Most methods require lengthy treatments and lack the simplicity needed for routine analysis. A more simple and rapid method is proposed by Verma et al. [54] based on the reaction with 2-iodylbenzoate in dilute acids that produces, almost instantaneously, an orange-yellow colour that reaches its maximum intensity within 1 min, at 444 nm. Recently, other alternative reagent, 2,2′-(1,4-phenylenedivinylene)bis-8-hydroxyquinoline (PBHQ) was proposed [87], its reacts with *p*-aminophenol to form blue complex, having a larger molar absorptivity than the published methods. Besides, the main advantage is the reduction of reagent consumption; the developed method is based on the microwave assisted alkaline hydrolysis of PCT to *p*-aminophenol (PAP) and its subsequent oxidation in alkaline medium by periodate to benzoquinoneimine, which reacts at room temperature with PBHQ to produce a blue indophenol dye. Also, a simple, rapid and specific method was developed for the determination of PCT and PAP [88]; it is based on the hydrolysis of PCT to PAP, which, using dissolved oxygen as an oxidant in the alkaline region, was further transformed into benzoquinoneimine, capable of reacting with tiron to produce a green indophenol dye. The stabilisation of indophenol dye was achieved by the addition of Cu(II) solution. The absorbance was measured at 601 nm in alkaline medium. In Table 1 are summarised other spectrophotometric methods.

2.1.2. Flow-injection spectrophotometric methods

Automated continuous-flow systems have contributed largely to the development of preliminary operations, as the versatility of these systems makes possible to automate non-chromatographic separation techniques, such as filtration, liquid–liquid extraction, gas diffusion, preparation and dialysis, frequently involved in the first steps of the analytical process. Recently, various flow-injection analysis along with suitable detection technique have also been reported for the determination of PCT. Growing interest in the use of FI in conjunction with UV/vis spectrometric techniques has been demonstrated in several papers [89–100]. Existing FIA methods for determining PCT are mostly based on the hydrolysis of paracetamol to *p*-aminophenol, which is then transformed into a coloured derivative by a suitable reaction. The derivatisation reaction based upon nitrosation, oxidation, diazo coupling and needed some special reagents. A number of reagents including 8-hydroxyquinoline (oxine) [39], sodium nitroprusside [60], *o*-cresol [40,59,90], sodium iodylbenzoate [89], 1,2-naphthoquinone-4-sulphonate [67], sodium salicylate [98], phenol [91], 1-nitroso-2-naphthol [90] and $NaNO_2$ [43,99] have been proposed for FI spectrophotometry. In this system the control of such reactions and/or manifolds is still complicated [99]. Besides, the cost of instrumentation may be prohibitive to many laboratories. Use of oxidant microcolumns in the FIA-spectrophotometric determination of PCT is proposed; the oxidant was immobilised on an ion-exchange resin [100]. Verma et al. [89] describe a method based on its oxidation with 2-iodylbenzoate in acid medium to produce a yellow-orange compound, which is monitored at 445 nm. Bouhsain et al. [39] propose a method based on the on-line alkaline hydrolysis of PCT to *p*-aminophenol and the reaction with 8-hydroxyquinoline in the presence of KIO_4 as oxidant to form a blue indophenol dye which absorbs at 608 nm. Previous batch studies using closed PTFE reactors demonstrate that PCT can be hydrolysed quantitatively to PAP in only 2 min using an irradiation power of 990 W and 5 M NaOH or in 3 min using 1100 W and 3 M HCl. The on-line alkaline hydrolysis, carried out in a 6 m reaction coil located inside the cavity of a Microdigest 301 system provides yield of 54.1% for a reaction time of 94 s and allows the complete automation of the analytical procedure. Also, Criado et al. [92] reports a continuous-flow-based spectrophotometric

Table 1
Determination of paracetamol by spectrophotometric methods

Reagent	Method	Reference
KIO_4	Oxidation in 3 M H_2SO_4 medium; $\lambda = 410$ nm	[55]
Ceric ammonium nitrate	$\lambda = 355$ nm	[56]
3-Methyl-2-benzothiazolinone hydrazone in the presence of ceric ammonium sulphate	In alkaline medium and extraction into $CHCl_3$; $\lambda = 535$ nm	[57]
Ce(IV)	For 90 min, in concentrated sulphuric acid in a water bath at 80 °C; $\lambda = 410$ nm	[58]
o-Cresol		[59]
Sodium nitroprusside	Paracetamol and phenacetin were hydrolysed by refluxing with 40% HCl	[60]
Iodylbenzene	Oxidation in acetona; $\lambda = 430$ nm	[61]
Potassium dichromate	Oxidation for 15 min in 6 M sulphuric acid at 80 °C; measurement of Cr(III) formed at 580 nm	[62]
Potassium dichromate	Reaction of the hydrolysed product of PCT with the reagent to form a stable violet coloured chromogen; $\lambda = 550$ nm	[63]
m-Cresol	Oxidative coupling	[64]
Potassium persulphate	In alkaline medium at 40 °C, for 10 min, $\lambda = 315$ nm	[65]
Peroxydisulphate	Kinetic method; in alkaline medium; $\lambda = 315$ nm	[66]
Sodium 1,2-naphthoquinone-4-sulphonate and cetyltrimethyl ammonium bromide	In alkaline medium; determination of hydrolysis products of paracetamol and phenacetin; $\lambda = 570$ and 500 nm, respectively	[67]
Sodium sulphide and Fe(III) or Ce(IV)	At ambient temperature; determination of *p*-aminophenol and acetaminophen by formation of a methylene blue like dye; $\lambda = 550$ nm	[68]
Pyrochatechol violet	Under basic conditions to form a coloured ion-pair complex; $\lambda = 652$ nm	[69]
Bromine water in aqueous medium and in presence of cationic, anionic and non-ionic micelles in aqueous and non-aqueous media	Development of a reddish-brown colour at a pH 8.9–9.3, maintained by 1% borax solution; $\lambda = 450$ nm	[70]
Bromate–bromide mixture	Kinetic analysis; generation of bromine; determination of paracetamol and acetylsalicylic acid in mixtures; applying partial least-squares regression to the kinetic photometric data	[71]
S^{2-} in the presence of Fe(III)	Microwave assisted alkaline hydrolysis; $\lambda = 540$ nm; in only 1.5 min under radiation power 640 W	[72]
Ammonium molybdate	In strongly acidic medium to produce molybdenum blue; $\lambda = 670$ nm	[73]
Ammonium molybdate	$\lambda = 695$ nm	[74]
3-Cyano-*N*-methoxypyridinium perchlorate + methoxyethanol + chloramine T	$\lambda = 448$ and 476 nm	[75]
Methanol	$\lambda = 248$ nm	[76]
pH-induced spectral changes	$\lambda = 262.5$ nm	[77]
4-Nitrosoantipyrine	Heating in strongly alkaline medium give a red colour; $\lambda = 515$ nm	[78]
Potassium ferrcyanide	Oxidation in 1 M NaOH; $\lambda = 480$ nm	[79]
Fe(III) in the presence of ferrozine	$\lambda = 562$ nm	[80]
Silver gelatine or auric complexes	Turbidimetric determination; in alkaline medium at room temperature after 15 min; $\lambda = 414$ and 539 nm, respectively	[81]
Fe(III) and 1,10-phenantroline	Simultaneous determination of PCT and PAP by using the H-point standard addition method	[82]
Tris(2,2′-bipyridine)–iron(III) complex in acid medium	Stopped-flow method for the determination of paracetamol and oxyphenbutazone	[83]
Absorbance difference method	Simultaneous and separate estimation of paracetamol and diclofenac sodium; $\lambda = 230$ and 254 nm and $\lambda = 260$ and 292 nm, respectively	[84]
Two-wavelength	Simultaneous determination of paracetamol and chlorzoxazone	[85]
Solvent extraction and acid–dye method with thymol blue at pH 3	Determination of paracetamol, salicylamide and codeine phosphate	[86]

method for the determination of PCT. Dilute samples containing PCT are continuously hydrolysed in an alkaline medium, using a household microwave oven, to *p*-aminophenol, which reacts with *o*-cresol in 3.5 M NaOH. The blue derivative thus formed exhibits an absorbance maximum at 620 nm. The development of continuous and stepwise automatic dilution and calibration system that enable analyte dilution and enhancement in multicomponent analyses are described by Dunkerley and Adams and applied to the simultaneous determination of paracetamol, caffeine and aspirin [93]. The continuous system has been used with a robot and the stepwise configuration integrated into a FIA system. All analyses are performed using a UV/vis diode-array detector and data analysis performed using principal components regression.

Direct determination of analytes based on intrinsic absorbance measurements in the UV region usually shows problems arising from the non-specific absorption, interferences becoming too frequent and so limiting its application. Recently, the use of solid-phase spectroscopy (SPS) has made possible the direct UV spectrophotometric determination of analytes in the

presence of other species also absorbing in this region and showing a strong spectral overlap without any prior separation and without suffering interference from them. On the other hand, the combination of FIA with SPS has made possible to automate SPS in both non-destructive spectroscopic techniques: spectrophotometry and spectrofluorimetry. This SPS–FIA integration, in which a surface (usually an ionic exchanger) is surrounded by a flowing stream and its interaction with radiation is monitored, belongs to the so-called flow-through optosensors. According to the number of analytes that a sensor can monitor from the same sample, sensors can be classified into two categories: (a) single-parameter sensors when they are sensitive to a single analyte or a family of chemical compounds (overall screening determination) and (b) multiparameter sensors when they can respond to several analytes.

In this way, various methods have been proposed; a simple flow-through UV optosensing device was developed for the determination of PCT based on its transient retention and concentration on a suitable active solid support (Sephadex QAE A-25 anion-exchange resin) packed in the flow cell and the continuous monitoring of its native absorbance on the solid phase at 264 nm [94]. The sample was injected into a 0.08 M NaCl carrier stream at pH 11.0 by using a simple monochannel FIA manifold. After developing the analytical signal, PCT was desorbed from the solid support by the carrier solution itself. A strong increase in sensitivity as well as a very much higher selectivity was achieved as compared with the conventional FI method as a consequence of the separation of the analyte from the sample plug and its retention on the active solid support placed in the detection area. A continuous and simple multiparameter sensor for the sequential determination of salicylamide and paracetamol by solid-phase UV spectrophotometry is described by Ruiz Medina et al. [95]. The sample containing these compounds is injected and then they are concentrated on-line on to an anionic exchanger (Sephadex QAE A-25) packed in a flow-through cell and its absorbance measured continuously at 300 nm. The procedure does not require any separation step and the sensor can be regenerated by the carrier itself. Also, Ruiz Medina et al. [96] propose a photometric biparameter sensor based on the sequential retention on an active solid support and on-line solid-phase detection of two analytes just by using two alternate carriers (at different pH values) in which the sample is sequentially injected, the carriers themselves being the regenerating agents of the solid support, respectively. That is, the same sensing solid-phase zone responds each time to only one analyte in the sample after its previous conditioning by passing the appropriate carrier/self-eluting solution. Ascorbic acid (AA) and paracetamol (two very used active principles either solely or in combination in commercial pharmaceutical preparations) are directly determined in this paper using a sequential photometric flow-through sensor with a dextran type anion exchanger resin as solid sensing zone and two alternate buffer solutions at pH 5.6 (for AA determination) and pH 12.5 (for PCT determination) as carrier/self-eluting solutions, respectively. Neither derivative reaction nor prior separation are necessary because of their intrinsic absorbance is used as analytical signal. Other continuous UV photometric flow-through biparameter-sensing device has been developed for the simultaneous determination of PCT and caffeine at 275 nm [101]. The sensor is based on temporary sequentiation in the arrival of the analytes to the sensing zone by on-line separation using C_{18} bonded phase beads placed into a minicolumn just before the flow cell. The sample containing are injected into the carrier solutions, PCT is determined first because it passes through the minicolumn, while CAF is strongly retained in it. Then, CAF is conveniently elated from the pre-column and develops its transitory signal. A single triparameter flow-through sensor with UV detection is described in other study for the simultaneous determination of acetaminophen, acetylsalicylic acid and caffeine [102]. The use of a solid phase (C_{18} silica gel) placed in an on-line microcolumn provides the sequential arrival of the analytes to the detection solid zone (also C_{18} silica gel beads placed in a flow cell), making possible the determination with only one injection. Also, for the first time, a multiparameter-responding flow-through system with solid phase UV spectrophotometric detection is described for the simultaneous determination of a mixture of three active principles (PCT, CAF and propyphenazone) using univariate calibration [103]. Quantitation is based on the direct UV absorbance measurements of the analytes when they reach the sensing zone (C_{18} silica gel) placed in the flow cell of the FIA system. Because of the strong spectral overlap showed by these analytes, a temporary sequentiation in their arrival to the sensing zone is required from one only injection. It is achieved by means of an on-line simultaneous retention of two of them (caffeine and propyphenazone) on a minicolumn filled with C_{18} silica gel placed before the solid-phase UV transductor, while PCT passes through the minicolumn and develops its transitory signal. Then, CAF and propyphenazone are successively eluted from the column using two different methanol/water solutions and reach sequentially the sensing zone, develop their respective signals.

Of all the FI procedures, the FI spectrophotometric determination of PCT by the oxidation of the analyte with potassium hexacyanoferrate(III) in basic medium followed by the reaction of the *N*-(hydroxyphenyl)-*p*-benzoquinomine, intermediate metabolite, with phenol giving a blue product, formulated as *N*-(*p*-hydroxyphenyl)-*p*-benzoquinonime as described by Calatayud et al. [97] seems a well-defined and simple method for the rapid determination of PCT. The main draw back of many of the FIA methods is however the problem of high consumption of the sample and especially reagents that makes the technique rather expensive.

Since its introduction in the early 1990s, sequential injection analysis (SIA) has become a widespread technique employed in many branches of chemistry. This technique has received a considerable attention and great acceptance owing to its simplicity, versatility, low sample and reagent consumption and its low cost. In contrast to FIA, SIA employs a computer-controlled multiposition valve and a pump operated synchronously. This enables a system to perform determinations of different analytes employing a single channel manifold with minor modifications. Further advantages are the precise manipulation of the stack of zones throughout the manifold system, e.g. stopping the penetrated zone in a water bath at elevated temperatures in a

more convenient way. van Staden and Tsanwani [104] propose a simple and inexpensive sequential injection analysis technique for the determination of PCT based on the oxidation reaction of PCT with potassium hexacyanoferrate(III) followed by the reaction with phenol at elevated temperature in aqueous ammoniacal solution with the main aim the reduction of reagent consumption. A simple FI and two different SI systems have been investigated for the determination of PCT by employing a simple reagent for a nitrosation reaction [105]. It is based on the on-line nitrosation of PCT with sodium nitrite in an acidic medium. The formed nitroso derivative species reacts further with sodium hydroxide to convert it to a more stable compound. The yellow product is continuously monitored at 430 nm. Also, the determination of PCT was carried out by reacting PCT with nitrous acid generated in-line and the product yielded was measured at 430 nm in alkaline medium [106].

An automated FI manifold based on iterative change of the flow direction has been designed to carry out continuous liquid–liquid extraction without phase separation and hydrolysis both with ultrasound-assistance [107]. The dynamic approach has been applied to suppositories from which PCT has been extracted in this way into an aqueous phase and hydrolysed prior to reaction with *o*-cresol in the alkaline medium used as extractant. The strategic location of the photometric flow cell in the FI manifold enables monitoring of the overall process and the obtaining of a characteristic multipeak recording. The influence of ultrasounds on the different steps was investigated. The indophenol blue dye formed was monitored at 620 nm.

2.1.3. Multivariate spectrophotometric methods

Computer-controlled instrumentation and multivariate calibration methods are playing a very important role in the multicomponent analysis of mixtures by UV/vis spectrophotometry. Both approaches are useful for the resolution of band overlapping in the quantitative analysis. In general, a multivariate calibration model is constructed from instrumental response data collected for a set of multicomponent samples of known concentrations with respect to the analytes of interest. Each method needs this calibration step, followed by a prediction step in which the results of the calibration are used to determine the component concentrations from the sample spectrum. In recent years, multivariate calibration techniques have been widely applied to UV/vis spectral data [108,109], classical least-squares (CLS), inverse least-squares (ILS) and methods such as principal components regression (PCR) and partial least-squares (PLS) are increasingly being used in conjunction with FI techniques. PCR and PLS are indirect calibration methods, i.e. they do not require individual spectra of each analyte and interferents to be known in advance. They, however, require the analysis of a comprehensive set of calibration samples that span all the expected physical and chemical phenomena that may influence the spectra of samples for prediction. Within this calibration set, the analyte concentration must be known, but the levels of any interference do not need to be known. This is in contrast to direct multicomponent analysis (DMA). In PCR, the spectra are decomposed on the basis of the maximum variance between spectral data without using information about the concentrations, whereas PLS uses both the spectral and concentration data in modelling. Hence, PLS sacrifices some fit of the spectral data relative to PCR in order to achieve better correlations to concentrations during prediction. PLS is a powerful multivariate statistical tool that has been successfully applied to the quantitative pharmaceutical analysis by using UV, NIR, fluorometric, Fourier transform infrared attenuated total reflectance and polarographic data.

Paracetamol, acetylsalicylic acid and caffeine are active principles widely used and frequently combined in pharmaceutical preparations. The simultaneous determination of these three active components and other additional ones can be performed by using techniques such as spectrophotometric, having proposed various numerical methods such as Kalman filtering of UV data [110], multivariate programs using spectrophotometric data such as multicomponents analysis, Simplex and Multic [111], factor analysis [112], iterative-target transformation factor analysis [113] and stepwise regression method [114]. However, still now, no attempt was made to use PLS for the simultaneous determination of the aforementioned compounds. A fast analytical procedure is proposed for the simultaneous determination of these compounds by means the PLS treatment of the spectrophotometric absorbance data between 216 and 300 nm, taken at 5 nm intervals [115]. The method involves the use of 8 standard mixtures of the three compounds assayed. Also, PCT, chlorpheniramine and pseudoephedrine were determined by CLS and PLS [116].

LS method in matrix form which is *K*-matrix representation of Beer's law is presented for simultaneous determination of ibuprofen and paracetamol without prior separation from each other [117]. The concentration of each component in the mixture was determined spectrophotometrically from absorbances of the mixture measured at 225, 226, 228, 232, 230, 234 and 235 nm. The UV spectrophotometric analysis of a multicomponent mixture containing paracetamol, caffeine, tripelenamine and salicylamide by using multivariate calibration methods, such as PCR and PLS, was described by Ragno et al. [118]. The calibration set was based on 47 reference samples, consisting of quaternary, ternary, binary and single-component mixtures, with the aim to develop models able to predict the concentrations of unknown samples containing as many as one-to-four components. The calibration models were optimised by an appropriate selection of the number of factors as well as wavelength ranges to be used for building up the data matrix and excluding any information about the interfering excipients included in pharmaceutics. The use of a solid support in combination with chemometric techniques provides a high selectivity and an increase in sensitivity [119]. This paper reports on the resolution of the ternary mixture of PCT, caffeine (CAF) and acetylsalicylic acid (ASA) and its simultaneous determination by PLS flow-through multisensor. Few studies have been reported about the simultaneous determination of analytes by FI spectrophotometry in solid phase. One of the most promising aspects of this type of sensors is the possibility of performing multicomponent analysis using photometric diode-array detector to monitor simultaneously the absorbance at various wavelengths or record the entire spectra in a fraction of a second. This paper deals with the use of the sorbing retention for the optosensing of the three analytes and their subsequent

determination by exploiting not only their spectral features on the solid support with the aid of a multicalibration chemometric approach but also their different kinetic behaviour in the retention–elution process onto the solid phase by selecting the spectra at two adequate time values which provides the most different behaviour by themselves.

The use of multivariate spectrophotometric calibration is reported for the analysis of two decongestant tablets, where PCT is the principal component and diphenhydramine or phenylpropanolamine are the minor components [120]. The resolution of these mixtures has been accomplished without prior separation or derivatisation, by using PLS-1. Kinetic methods are often useful for the determination of multicomponent mixtures. Most commonly, the selectivity of these multicomponent determinations arises from the different rates at which components of a mixture react with a common reagent, and it has made a great improvement by using the chemometric procedures. Various methods, including proportional equations, curve resolution, Kalman filter and CLS have been applied in this field to resolve the multicomponent kinetic systems. Recently, the chemometric methods based on factor analysis and artificial intelligence, including PCR, PLS and artificial neural networks (ANN), have found increasing applications for multicomponent kinetic determination, which do not require prior knowledge of reaction order or reaction rate coefficient of the involved analytical systems. These methods make it possible to eliminate or reduce the effects of the analyte–analyte interaction, the synergistic effect (non-additivity of reaction rates), the multistep process and any other unknown non-linearity. In these chemometric works, generally, a set of calibration samples with known compositions is first prepared and the kinetic measurements are carried out, after which, the mathematical models are established by processing the measured kinetic data. Subsequently, the mathematical models are used for the prediction of unknown samples under the same experimental conditions. Very recently, the application of an ANN model that uses as input data the scores of a principal component analysis model (PC-ANN) to quantify mixtures in different kinetic situations has been reported.

Bozdogan et al. [121,122] discussed the simultaneous spectrophotometric determination of acetaminophen and phenobarbital and paracetamol, metamizol sodium and caffeine, respectively, with the aid of PLS. Ni et al. [123] presents an alternative method based on kinetic spectrophotometry for the simultaneous determination of acetaminophen and phenobarbital with the aid of chemometric approaches. The method relies on the oxidative coupling reaction of these two compounds with 3-methylbenzothiazolin-2-one hydrazone (MBTH). The capability of MBTH to form colour products with a number of organic compounds, especially pharmaceutical compounds in the presence of a suitable oxidant, has given rise to the development of numerous analytical methods. In this work, the reaction of binary mixtures of the two pharmaceuticals with MBTH in the presence of hydrochloric acid and the Fe(III) oxidant was investigated. The absorbance spectrum at each reaction time was measured and recorded, and all these data were processed by ANN with and without the pre-treatment of PC-ANN and PLS.

The tri-linear regression–calibration (TLRC) and multilinear regression–calibration (MLRC) were developed for the multiresolution of ternary mixtures of CAF, PCT and metamizol (MET), and PCT, ascorbic acid and ASA, which have closely overlapped in the spectra [124,125]. The calibration algorithms were briefly described for the three-component systems, CAF–PCT–MET and PCT–AA–ASA, respectively. The data treatments were carried out by the Maple V, Excel and SPSS 10.0 Softwares.

In the last decade, three-way or, more generally, *N*-way analysis was introduced in the field of analytical chemistry. A three-way array may be obtained by collecting data tables with a fixed set of objects and variables under different experimental conditions, such as sampling time, temperature, pH, etc. The tables collected under various conditions can be stacked providing a three-dimensional arrangement of data. In some situations, even higher dimensional arrays may be considered. These methods can be applied for exploratory analysis, curve resolution, analysis of variance and calibration purposes using spectrophotometric, spectrofluorimetric, chromatographic, FI, sensory analysis or experimental design data. For *N*-way multivariate calibration, *N*-way partial least-squares (N-PLS) has recently received more attention than other tools. In this way, a procedure was proposed for determination of ASA, PCT and CAF based on multivariate calibration and UV spectrophotometric measurements (210–300 nm) [126]. The calibration set was constructed with nine solutions in the concentration ranges from 10.0 to 15.0 $\mu g\,ml^{-1}$ for ASA and PCT and from 2.0 to 6.0 $\mu g\ ml^{-1}$ for CAF, according to an experimental design. The procedure was repeated at four different pH values: 2.0, 3.0, 4.0 and 5.0. PLS models were built at each pH and used to determinate a set of synthetic mixtures. The best model was obtained at pH 5.0. An *N*-way PLS model was applied to a three-way array constructed using all the pH data sets and enabled better results. Finally, a new and very simple method was developed for the simultaneous determination of binary and ternary mixtures, without prior separation steps [127]. This method is based on the mean centering of ratio spectra. This method eliminates derivative steps and therefore signal-to-noise ratio is enhanced. After modelling procedure, the method has been applied to the simultaneous analysis of mixtures of mefenamic acid (MEF) and PCT and mixtures of ASA, AA and PCT.

2.1.4. Derivative spectrophotometric methods

The main problem of spectrophotometric multicomponent analysis is the simultaneous determination of two or more compounds in the same mixtures without preliminary separation. Several spectrophotometric determination methods have been used for resolving mixtures of compounds with overlapping spectra. When these methods are compared with each other, the range of application of derivative spectrophotometry is more reliable with respect to utility and sensitivity than normal spectrophotometry. Table 2 summarises the use of derivative methods to the determination of PCT in mixtures with other compounds.

Table 2
Determination of paracetamol by derivative spectrophotometric methods

Other components	Remark	Reference
Acetylsalicylic acid	By the linear absorbances method and the derivative	[128]
Acetylsalicylic acid	First derivative; comparison with HPLC	[129]
Analgine	First derivative spectrophotometry and absorbancy ratio method in the zero-order spectra	[130]
Ascorbic acid	First derivative	[131]
Caffeine	First derivative; comparison with HPLC	[132]
Caffeine	Comparative study of the ratio spectra derivative spectrophotometry, Vierordt's method and high performance liquid chromatography (HPLC)	[133]
Codeine	First derivative using the graphical and zero-crossing methods	[134]
Dipyrone	First derivative using a zero-crossing technique	[135,136]
Hyoscine *n*-butyl bromide(I)	By precipitating (I) with ammonium at pH 6.0 and reading the absorbance of the solution of the precipitate in acetone at 525 nm and first derivative for PCT	[137]
Mefenamic acid	Ratio spectra derivative spectrophotometry and chemometric methods	[138]
Mephenoxalone	Third derivative using a zero-crossing technique	[139]
Methocarbamol	Ratio spectra derivative spectrophotometry and HPLC	[140]
Methocarbamol	Second derivative	[141]
Phenprobamate	First derivative at different wavelengths	[142,143]
Phenprobamate	Derivative-differential UV spectrophotometry based on pH changes and ratio-spectra first derivative spectrophotometry	[144]
Pheylpropanolamine hydrochloride	First derivative by measuring the absorbances between 215 and 280 nm	[145]
Potassium acesulpham	Comparison of two methods: absorbance at isosbestic wavelength and first derivative	[146]
Propacetamol	First derivative; measurements are made at the zero-crossing wavelengths	[147]
Acetylsalicylic acid and ascorbic acid	Double divisor-ratio spectra derivative and ratio spectra-zero-crossing methods	[148]
Acetylsalicylic acid and caffeine	Application of derivative and continuous wavelet transfers to the overlapping ratio spectra	[149]
Caffeine and metamizol	Based on the use of the derivative of the ratio spectrum obtained by dividing the absorption spectrum of the ternary mixture by a standard spectrum of a mixture of two of the three compounds in the title mixture	[150]
Caffeine and propyphenazone	Derivative ratio spectra-zero-crossing spectrophotometry and HPLC	[151]

2.2. IR spectrophotometric methods

2.2.1. NIR spectrophotometric methods

Quality control analyses in the pharmaceutical industry involve the determination of multiple parameters for both raw materials and end products. NIR spectroscopy has proved to be a powerful analytical tool for analysing a wide variety of samples used in the agricultural, food, petrochemical, textile and pharmaceutical industries, especially the use of NIR spectroscopy for quantitative analysis of the pharmaceutical tests has experienced a significant increase during the two last decades. Currently, the quantitative analytical methods of pharmaceutical, such as UV spectrophotometric, GC and HPLC methods, usually require dissolving the samples, separating them and determining their ingredients. However, NIR technique is a rapid and non-destructive quantitative analytical technique of the samples with no need for reagents or solvents. The high industrial interest aroused by NIRS in recent years is a direct result of dramatic advances in this technique including the increasing availability of fast-scan instruments that enable measurements with little or no sample manipulation and of software for implementing powerful chemometric techniques. The NIRS technique has proved a highly useful tool for controlling powdered pharmaceuticals, which it can readily identify and quantify. The ability to analyse intact dosage forms has dramatically facilitated monitoring of every single step in the production process as well as of the final form, all with minimal sample manipulation. The earliest applications of NIRS to intact tablets were reported in 1988; the whole surface of a double-reflecting Al sample container was illuminated for the qualitative analysis of tablets. Later applications have been primarily concerned with qualitative analyses. Most reported direct NIRS analyses of tablets are subject to two hindrances, namely: (a) the lack of appropriate devices for making reproducible sample measurements and (b) the fact that measurements are made by reflectance, so the information acquired corresponds to the sample surface, which may lead to spurious results with coated tablets.

These shortcomings are circumvented by recently developed NIR instruments of improved design. Their measuring modules provide more reproducible and reliable measurements of individual tablets. Also, the new instruments use transmission measurements (i.e. the NIR beam passes through the whole sample thickness). Using these instruments provides a number of advantages, namely,

1. The ability to draw information for single tablets from their individual spectra.
2. The ability to analyse coated tablets, the coating thickness posing no special problem since the spectrum is due to the whole tablet.
3. Increased reproducibility in the measurements, those of individual tablets included, by virtue of the response being

produced by a sizeable amount of sample, its entire thickness.
4. Simple calibration models relative to those typically used with reflectance measurements, the recorded signal (absorbance) can be assumed to obey the Lambert–Beer law since the improved instrument design results in minimal scatter.

On the other hand, the most severe constraint on transmission measurements is posed by the thickness of typical tablets. Because the light beam must travel through a large amount of product, only a small fraction of incident light reaches the detector. The high absorbances involved in this type of measurement, most often in excess of 4 absorbance units, have compelled instrument manufacturers to improve existing commercial equipment by minimising losses of incident light on its way to the detector and using highly sensitive detectors affording readings of much higher absorbances than those usually recorded in other spectroscopic techniques. Near infrared transmittance spectroscopy was used to determine the analgesic PCT in a pharmaceutical preparation commercially available as tablets [152,153]. Spectra were recorded on a dedicated instrument that measures the transmission of intact tablets over the wavelength range 600–1900 nm. Spectral data were processed by using two multivariate calibration methods, viz. stepwise multiple linear regression (SMLR) and partial least-squares regression (PLSR).

In qualitative and quantitative analysis, artificial neural networks are more widely applied during the past several years. The better advantage of ANN is its anti-jamming, anti-noise and robust non-linear transfer ability. In proper model, ANN results in lower calibration errors and prediction errors. A method for simultaneous analysis of the two components of compound paracetamol and diphenhydramine hydrochloride powdered drug has been developed by using ANN–NIR spectroscopy [154]. An ANN containing three layers of nodes was trained. Various ANN models based on pre-treated spectra (first derivative, second derivative and standard normal variate, SNV) were tested and compared. PLS were also used, which were compared with ANN. The best model was obtained at first derivative spectra. Also, paracetamol and amantadine hydrochloride were simultaneously determined in combined paracetamol and amantadine hydrochloride tablets and powder by using near infrared (NIR) spectroscopy and ANNs [155]. The ANN models of three pre-treated spectra (first derivative, second derivative and standard normal variate, respectively) were established.

NIR spectroscopy was used to determine paracetamol, caffeine and lactose based excipient content of powders for direct compression and in intact tablet formulations as well [156]. The nominal concentrations of the active moiety were different in each sample set, and calibration was carried out by multiple linear regression calculations from the reflectance spectral data. The non-destructive NIR method applied is thus suitable for the alternative quantitative determination of PCT, CAF and additionally the excipient in tablets and has the advantage over HPLC of being rapid and can be simply carried out without sample preparation and without the use of any reagent.

NIRS meets many such requirements, so it has grown substantially in use as a quality control technique in the pharmaceutical industry. In fact, the NIRS technique provides a number of attractive advantages, namely:

(a) It allows direct recording of spectra for solid/liquid forms with little/no sample pre-treatment.
(b) It allows chemical and physical information about samples (e.g. viscosity, moisture content, polymorphism) to be derived from spectra.
(c) It affords multiparameter determinations from a single spectrum.
(d) It can be used with various spectral recording modules compatible with virtually any type of working site and operating procedure.

On the other hand, the NIR spectroscopy has two major disadvantages, namely:

(a) NIR spectra exhibit strong band overlap, which requires the use of multivariate chemometric techniques in both qualitative and quantitative analytical applications.
(b) The low sensitivity of the technique restricts its scope to major components and a few minor components at most.

The use of multicomponent analytical methods in general and NIR spectroscopy in particular, can substantially improve the analytical control of production processes by shortening analysis times and improving quality as a result. While the vast majority of pharmaceutical preparations contain a single active principle, some include two or more at concentrations frequently spanning wide concentration ranges. The joint determination of all the active principles in a multicomponent formulation is made especially difficult by the frequent fact that some species are present at concentrations near the determination limit of the technique. A NIR spectroscopic method for the simultaneous determination of five active principles present in a drug for alleviating influenza symptoms (paracetamol, ascorbic acid, dextrometorphan hydrobromide, caffeine and chlorpheniramine maleate) was developed [157]. The method is intended to replace the current choice, which uses the HPLC technique for four of the analytes and iodometric titration for the fifth.

2.2.2. *FTIR spectrophotometric methods*

A Fourier transform IR (FTIR) spectrometric technique is described for the simultaneous determination of ibuprofen and paracetamol [158]. Quantification was carried out by measuring the absorbances at 1684 and 1740 cm^{-1} for paracetamol and ibuprofen, respectively, using the baseline established at 1780 cm^{-1} for measurement correction.

Work coupling FI and FTIR spectrometry first appeared about a decade ago. A procedure for the direct FTIR spectrometric determination of PCT is described [159]. The method is based on the solubilisation of paracetamol in a 10% (v/v) ethanol in CH_2Cl_2 solution and direct absorbance measurement at 1515 cm^{-1}, using the baseline established at 1900 cm^{-1} for measurement correction. The procedure can be carried out in

both the stopped-flow and FI modes. Also, a procedure is proposed for the simultaneous determination of PCT, ASA acid and CAF by PLS–FTIR in the wavenumber range 823 and 1775 cm^{-1}, using the 14 characteristic bands included in this interval for the three compounds under study [160]. The method involves the leaching of the compounds to be determined from the pharmaceutical formulations with a 10% (v/v) solution of ethanol in dichloromethane in an ultrasonic water bath for 20 min, filtration and direct introduction inside a liquid flow cell, taking 16 cumulated scans obtained in the stopped-flow mode. For calibration a reduced set of eight standard mixtures considered at two concentration levels, are required.

The application of the combined technique for quantitative determinations with the use of suitable flow cells to obtain information from transient IR signals has demonstrated its capability for the following:

1. The accurate measurement of the baseline and the exact determination of the absorbance maximum.
2. The simultaneous determination of various compounds in the same sample.
3. Pharmaceutical analysis.
4. Determination of organic compounds by the use of FTIR derivative spectrometry.

The analysis of aqueous solutions by IR spectrometry is usually accomplished by attenuated total reflectance (ATR). The mode of operation of the CIRCLE® accessory is based on the ATR technique. The FI/FTIR technique coupled with the CIRCLE® accessory provides a potential combination for rapidly analysing and quantifying pharmaceutical samples in aqueous solution. Optimisation of a basic FI–FTIR system using the CIRCLE® has been reported for the determination of acetaminophen in aqueous solutions [161]. All of these papers have reported on the use of infrared (IR) spectrometry for monitoring FI transients; however none of these describe applications involving on-line chemical derivatisation. Ramos et al. [162] presents an FI procedure using FTIR of aqueous solutions in which an on-line reaction product is monitored. Using an attenuated total-internal reflectance flow cell the new FI/FTIR method was developed to follow the alkaline hydrolysis of acetaminophen followed by the oxidation reaction of the hydrolysis product in aqueous media. This oxidation reaction was carried out at room temperature. There was no need for the oxidative coupling reaction with phenol at elevated temperature. The proposed method is based on the alkaline hydrolysis of the analyte to produce *p*-aminophenol and its oxidation reaction with potassium ferricyanide to produce *p*-benzoquinone-monoimine which eventually oxidises to form *p*-benzoquinone. The chemistry of the reaction was studied both, in the visible and IR regions of the spectrum and the method has been developed by the application of FI methodology. The micro-flow version of the CIRCLE® IR accessory, which is compatible with aqueous solutions, was used. Measurements were carried out at the OH-phenolic deformation (1274.1 cm^{-1}) and the aromatic ring mode (1498.2 cm^{-1}) infrared vibrations for the hydrolysis product, *p*-aminophenol.

There is an ever-growing interest for fast, simple and reliable analytical methods for the determination of active pharmaceutical substances in the solid state. Such methods become more attractive when two substances co-exist in the same commercial dosage form or two polymorphs of the same compound are present in mixture. IR spectroscopy is a widely recommended method, wherein the spectrum of the test compound is compared with that obtained concomitantly of the reference standard. Recently FT-Raman spectroscopy has been suggested as a capable method for identifying polymorphs and testing crystallinity of substances.

For PCT (acetaminophen, 4′-hydroxy acetanilide) two polymorphs are fully described in the literature: the monoclinic, and the orthorhombic. A third form has been reported, but is very unstable. The monoclinic form is thermodynamically stable and is used commercially, but it lacks slip planes and therefore it is not suitable for direct compression into tablets. Orthorhombic paracetamol is characterised by well-developed slip planes in its crystal structure and undergoes plastic deformation making it suitable for tabletting by direct compression. A simple, fast and reliable identification and quantification method for the orthorhombic paracetamol in powder mixes with monoclinic was thought of interest. FTIR and Raman spectroscopic methods are suggested for identification of orthorhombic and monoclinic paracetamol and for their quantitative determination in mixes [163]. Also, a procedure for quantitative determination of ASA and PCT by PLS and PCR treatment of FT-Raman spectroscopic data is proposed [164]. Three chemometric models were built: the first, for samples consisting of an active substance diluted by lactose, starch and talc; the second, in which a simple inorganic salt was applied as an internal standard and additions were not taken into account; and the third, in which a model was constructed for a commercial pharmaceutical, where all constituents of the tablet were known.

2.3. Spectrofluorimetric methods

Spectrofluorimetric methods with lower detection limits have been proposed for the determination of PCT [165–170]. Because PCT is not a fluorescent species, it can be determined indirectly using Ce(IV) [166] as an oxidising reagent and measuring the relative fluorescence intensity of Ce(III) arising from Ce(IV). Direct spectrofluorimetric determinations of PCT require a previous and adequate derivatisation step. Reagents such as fluorescamine and dansyl chloride have been proposed [167,168] but both reactions show low selectivity. 1-Nitroso-2-naphthol [169] and potassium hexacyanoferrate(III) [170] have been proposed as oxidising reagents, since an adequate oxidation of PCT produces fluorogenic species suitable for its determination. A FI spectrofluorimetric determination of PCT is reported, based on the oxidation of the analyte with potassium hexacyanoferrate(III) immobilised on an anion-exchange resin, the fluorescence being enhanced with *N*,*N*′-dimethylformamide [171].

A stopped-flow method with fluorescence detection for the determination of PCT based on its oxidation with hexacyanoferrate(III), is described [172]. A kinetic study of the reaction is developed measuring the initial rate of change of the

fluorescence intensity of the oxidised product formed at 241 and 426 nm excitation and emission wavelengths, respectively. Other alternative proposed is the application of first derivative fluorescence spectrometry to the simultaneous determination of paracetamol and salicylamide [173]. The method is based on the oxidation of the PCT with potassium hexacyanoferrate(III) and using a zero-crossing technique of measurement. Also, a spectrofluorimetrical method was designed for determination of PCT [174]. The employed methodology involves coumarinic compound formation obtained by reaction between PCT and ethylacetoacetate in the presence of sulphuric acid as catalyst. The reaction product is highly fluorescent at 478 nm, being excited at 446 nm.

As can be see, most of the reported procedures require a previous oxidation of PCT, which may increase the reagent consumption, time required for analysis and possibility of sample contamination. A sample preparation step prior to the determination makes the above-mentioned procedures somewhat laborious and less attractive for multisample analyses. This is often a limiting factor in the chemical and pharmaceutical industries, where a large number of samples must be assayed for quality control purposes. Simpler procedures performed in a shorter time are preferred and analytical procedures that do not involve use of chemicals and/or sample pre-treatment steps are then a good alternative. In this context, NIR [152,153] and FT-Raman [164] spectroscopy have been used for PCT determination in the solid phase. However, quantitative determinations with these techniques are only feasible by applying chemometric algorithms, especially in relation to NIR, where the spectra do not have high resolution, and complex-calibrating models must be constructed. Spectrofluorimetry in the UV/vis region can be used to perform the measurements directly in the solid matrix, leading to favourable characteristics of simplicity, sensitivity, ruggedness, selectivity, rapidity, etc. Non-destructive analyses are carried out that follow the present tendency towards Clean Chemistry. Moreover, the possibility of using optical-fiber accessories for on-line and/or in situ analysis becomes feasible. To date, this strategy has scarcely been exploited in relation to solid samples of pharmaceutical interest. Regarding PCT determination, a literature survey reveals that it usually requires the use of derivative reactions because PCT is not intrinsically fluorescent in aqueous solutions. In this regard, preliminary experiments demonstrated that PCT is fluorescent in the solid phase. The native fluorescence of PCT in the solid state is demonstrated [175]. It is easily adaptable to any spectrofluorimeter, and no chemical treatment of the sample is needed. The fluorescence measurements ($\lambda_{ex} = 333$ nm; $\lambda_{em} = 382$ nm) are performed directly on the powdered sample, the active substance being diluted in lactose, maize starch, poly(vinylpyrrolidone), talc and stearic acid.

2.4. Chemiluminescence methods

Due to its inherent high sensitivity and low detection limit, chemiluminescence (CL) has been applied to the analysis of many biomedical important analytes. The CL analysis can be measured from the CL induced by the reaction of analyte with CL reagents or the inhibition of CL that resulted from the reaction of analyte with CL reagents or oxidants prior to the CL reaction. Most of the reported procedures use the well-known luminol, peroxyoxalate or lucigenin CL reaction systems. Work on CL reactions of organic compounds formulated in pharmaceutical preparations is limited and an extensive evaluation of CL in drug analysis is not available. Recently, a certain number of pharmaceuticals have been determined through continuous-flow chemiluminescence procedures and at present it still shows a growing trend. A rapid and precise continuous-flow method is described for the determination of PCT based on the chemiluminescence produced by its reaction with Ce(IV) in acidic solution [176]. The system luminol–H_2O_2–$Fe(CN)_6^{3-}$ is proposed for first time for the indirect determination of PCT. The method is based on the oxidation of paracetamol by hexacyanoferrate(III) and the subsequent inhibitory effect on the reaction between luminol and hydrogen peroxide [177].

However, detection limits of 1.0 and 2.5 $\mu g\,ml^{-1}$ obtained by the above-mentioned CL analysis were not comparable with the high sensitivity and low detection limit characteristics of CL analysis. Development of a more sensitive CL detection procedure for PCT analysis should be of interest. By considering the fact that permanganate is an oxidant applicable in a luminol based CL system and paracetamol can be oxidised by it in a basic medium, an inhibition of CL in a luminol–$KMnO_4$ system should result from the introduction of PCT to a luminol–$KMnO_4$ system. A simple and effective procedure based on the reaction of paracetamol with $KMnO_4$ in a luminol–$KMnO_4$ system was, thus, developed for the CL analysis of paracetamol [178].

Tris(2,2′-bipyridyl)Ru(II) has been used as the basis of CL detection of a wide range of compounds after oxidation to the Ru(III) complex. The analyte interacts with the Ru(III) complex reducing it to the Ru(II) complex in an excited state, which then emits CL as it returns to the ground state. In this way, a FI procedure for PCT determination with CL detection was proposed in which Ru(II) was oxidised on-line by mixing with potassium permanganate solution. Subsequent reaction with PCT produces chemiluminescence. The intensity is enhanced by the presence of Mn(II) ions [179]. This work describes a relatively sensitive, rapid and reproducible FI–CL method for PCT determination based on tris(2,2′-bipyridyl)ruthenium(II) without sample hydrolysis process.

2.5. Determinations in biological samples

Paracetamol is readily absorbed after administration and widely distributed throughout most body fluids. Its metabolic pathway comprises conjugation to form glucuronide and sulphate derivatives. About 90% of the therapeutic dose is excreted in the urine in 24 h as conjugated derivatives, 1–4% of the excreted material being unchanged drug.

The determination of PCT and its metabolites in biological fluids (urine, plasma and serum) has usually been carried out using either chromatographic or electrophoretic techniques. In this regard, high performance liquid chromatography with UV/vis and electrochemical detection or nuclear magnetic resonance–mass spectrometry, high performance thin-layer chromatography and gas chromatography and capillary

electrophoresis have been extensively used. Conventional photometric, fluorimetric and electrochemical methods have occasionally been reported for the determination of PCT and its metabolites in biological fluids, as they are preferred for the determination of this compound in pharmaceuticals. A spectrofluorimetric method for the determination of paracetamol is presented, based on the oxidation of the analyte to give the fluorophore 2,2′-dihydroxy-5,5′-diacetyldiaminebiphenyl [180]. Sodium hypochlorite was used as an oxidising reagent. On the other hand, sample screening systems are focused on providing an expeditious reliable response in relation to a specific property of the sample, minimising preliminary operations, as well as, the need for permanent use of sophisticated instruments. Indeed, only those samples for which the screening system has previously provided a reliable positive response will be further processed following a conventional analytical method. The use of sample screening systems involves several advantages concerning reduction of costs, rapidity and simplicity. In this regard, the combined use of continuous-flow systems and an evaporative light scattering detector (ELSD) for screening purposes can provide several advantages such high versatility, simplicity and low cost.

Development of fast response analytical systems is of special relevance in clinical and toxicological laboratories dealing with a large number of samples each day that must be handled with the least possible delay. Urinary screening of PCT is usually carried out using either acid [181,182] or enzymatic [183,184] hydrolysis of the analyte to yield *p*-aminophenol; the hydrolysis product reacts with *o*-cresol in alkaline medium to form a coloured derivative, which can be monitored at 620 nm. Although the analytical reaction is fairly fast, the hydrolysis step is the major limitation to improving the sample throughput of this frequently ordered test, as it requires between 20 and 30 min for completion. Another major inconvenience of this method is the high interference observed for *N*-acetylcysteine (the drug of choice for the treatment of paracetamol overdose), thus leading to false negative results in urinary screening. This interference makes it necessary to carry out this determination in serum samples, where the results obtained are consistent with the dosage [182] although it will also be dependent on the assay followed.

There is growing interest in developing rapid response analytical systems devoted to the determination of clinical parameters, as the result obtained will condition the course of treatment to be administered to the patient. A sample screening system provides a binary yes/no response, which indicates if the target analytes are present above or below a pre-set concentration; their potential in analytical chemistry has been recently pointed out. The most favourable situation is when the screening method can be used with no sample pre-treatment. A sample screening system has been developed and it was aimed at avoiding the two major limitations of conventional methods for the screening of paracetamol and its metabolites in urine samples, namely the hydrolysis time and the interferences [185]. The fully automated method developed uses for the first time on-line acid microwave assisted hydrolysis of the drug, which is complete in 2.0 min, followed by the formation of a dye with *o*-cresol in NaOH medium. Under the proposed conditions, the most commonly addressed interfering compounds are tolerated at higher concentrations than in existing conventional methods.

No references regarding the use of an ELSD for the determination of paracetamol in biological fluids have been reported previously. The ELSD is well established as a universal detector for liquid and supercritical fluid chromatography, offering several advantages such as weakly dependence on optical properties of the analytes, sensitivity higher than that of the refractive index detector and compatibility with a mobile phase gradient. For these reasons, the ELSD has always been used as detector of a highly selective separation technique. The use of a simple FI system coupled on-line to an ELSD for sample screening purposes is described for the first time [186]. The configuration designed was applied to the direct and automatic screening of biological fluids for paracetamol and its metabolites, avoiding both the hydrolysis and derivatisation steps. A miniaturised sorbent column was included for matrix clean-up and analyte pre-concentration. The interface between the flow system and the detector was constructed by using a high pressure six-port injection valve. Aliquots of a few microlitres of diluted urine or serum can be directly injected into the system, the analytical signal being obtained in <3 min.

The diagnosis and treatment of PCT overdose are based largely on the plasma concentration and the time of drug ingestion. It is recommended that the overdosed patients must be given an antidote within 10 h of ingesting the drug. Thus, an accurate history of the time of ingestion is of critical importance and to obtain this information, rapid quantitation of PCT in the serum is necessary. The clinical laboratory, therefore, plays a critical role in the confirmation and treatment of PCT poisoning. It must be able to provide the clinicians with accurate serum PCT levels on an emergency basis. There are many reliable laboratory methods for assaying serum acetaminophen levels, but they are often time-consuming, technically demanding and requires the use of costly, highly specialised instruments. Afshari and Liu [187] have developed a quick, economical assay spectrophotometric method for PCT. The proposed method is based on the sodium periodate-catalysed oxidative coupling of acetaminophen-derived *p*-aminophenol and *p*-xylenol via an electrophilic substitution reaction. Other proposed methods for determination of PCT in biological fluids were summarised in Table 3.

3. Electroanalytical methods

Techniques typically employed in clinical laboratories encompass titrimetry, chromatography, spectrophotometry and immunoassays. Generally, the analysis times are lengthy and the suitability for routine bedside measurements is compromised by the necessity for dedicated and cumbersome analytical equipment. Amperometry is an electroanalytical interfacial technique, which, when used in conjunction with disposable sensors, has many inherent advantages that address these limitations. The most striking features of amperometric sensors are their simplicity, low cost and amenability to miniaturisation. The drawback associated with this approach is that any compound that rapidly exchanges electrons with the electrode will, theoretically, be

Table 3
Proposed methods for determination of paracetamol in biological fluids

Sample type	Method	Reference
Biological specimen	Colorimetric method based on the measurement of crimson colour produced when PCT is treated with 10% NaOH	[188]
Serum and plasma	Colorimetric procedure	[189]
Plasma and other biological fluids	Extraction, dissolution of the residue, hydrolysis, spectrofluorimetry (excitation and emission at 280 and 370 nm, respectively)	[190]
Blood	Elimination of salicylate interference in the UV determination	[191]
Serum	Visible spectrophotometric; extraction; reaction with $Fe(NO_3)$-2,4,6-tris(2-pyridyl)-*s*-triazine; $\lambda = 593$ nm	[192]
Human serum and pharmaceutical formulation	Simultaneous spectrophotometric determination of PCT and salicylamide; differential kinetic method; oxidation by Fe^{3+} in the presence of 1,10-phenantroline as indicator	[193]
Blood serum	By first derivative UV absorption spectroscopy	[194]
Urine	By first hydrolysing the glucuronide and sulphate metabolites with β-glucurinidase, extraction and, finally, TLC, fluorescent quenching and densitometry	[195]
Human urine	A series of clean-up columns for solid-phase extraction were packed with various C_{18} phases of different surface properties; these columns were used for the isolation and determination of PCT and its metabolites	[196]
Urine	Second-order derivative UV spectrophotometric; direct determination	[197]
Urine of HIV+/AIDS patients	Validated method for determination of paracetamol and its glucuronide and sulphate metabolites by wavelength-switching UV detection	[198]

detected. Specificity may be attained by deliberate tailoring of the sensors' response characteristics with a host of modifying agents.

Paracetamol can be readily oxidised at a carbon paste or glass carbon electrodes, but these amperometric procedures are non-selective, since the potential involved in this process ranges from 0.6 to 0.8 V and various substances are electroactive in this potential interval. However, the application of biosensors for this task, can be operated at potentials much lower than those normally used, thus decreasing the interference.

Amperometry has been used for determining paracetamol in liquid chromatographic eluates [199] and directly in whole blood [200,201]. The principle of both methods involved the measurement of the product of the enzymatic hydrolysis of paracetamol catalysed by aryl acylamidase. The product, *p*-aminophenol, has a lower oxidation potential than the parent compound and hence lowers the susceptibility of the system to interfering compounds that would elicit a response at the sensor surface if higher voltages were applied. Vaughan et al. [201] achieved additional specificity by the juxtaposition of a glutathione (GSH)-impregnated paper over the paracetamol-sensing enzyme strip. The GSH layer serves to sequester endogenous thiols that would otherwise hamper analyses by their 1,4-Michael reaction with the benzene ring in the drug, generating new electroactive compounds.

The performance of a thin-layer flow detector with a glassy carbon electrode coated with a film of protonated poly(4-vinylpyridine) is described [202]. Substantial improvement in the selectivity of amperometric detection for liquid chromatography and flow-injection systems is observed as a result of excluding cationic species from the surface. The detector response was evaluated with respect to flow rate, solute concentration, coating scheme, film-to-film reproducibility and other variables. Protection from organic surfactants can be coupled to the charge exclusion effect by using a bilayer coating, with a cellulose acetate film atop the poly(4-vinylpyridine) layer. Applicability to urine sample is demonstrated.

Amperometry and/or voltammetry are electroanalytical techniques which can be used in conjunction with disposable sensors and/or biosensors showing inherent advantages as simplicity, low cost and rapidity. Biosensors are devices that combine higher selectivity and sensitivity of a biological component with a suitable transducer. The biological sensing element, usually an enzyme or an antibody, recognises the complementary molecule and the resulting biochemical changes are transduced into a proportional concentration signal.

Gilmartin and Hart [203] development of a disposable, amperometric sensor for the rapid testing of urine paracetamol, based on surface-modified, screen-printed carbon electrodes (SPCEs). The benefits of the present CA-SPCE ensemble lie in their simplicity, low cost and selectivity. Additionally, as labile biocatalytic recognition components have been omitted in the sensor design, the operational lifetime and storage properties are likely to be advantageous. The transducer is a reagentless, amperometric sensor that was developed through the combined use of screen-printing and permselective membrane technologies. The sensor operates selectively by means of an anti-interference barrier, cellulose acetate, which is drop-coated directly on to the screen-printed carbon electrodes. The disposable, amperometric sensor requires no further modification procedures to enhance selectivity, i.e. no enzymes are involved, and hence their fabrication is simple and extremely economical.

Amperometric detection with two polarised indicating electrodes is based on the measurement of the current passing through two identical, usually inert electrodes to which a small potential difference is applied. The current flowing in the detection cell is observed only when the solution contacting the electrodes contains two forms of the reversible redox couple. Biamperometric detection has found applications in

flow-injection analysis. This combination offers the advantages derived from the selectivity of biamperometric detection due to low applied potentials and increased sensitivity and injection rate from the inherently lower dispersion of the FIA methodology. Several methods have been proposed for the determination of paracetamol based on FIA. Electrochemical methods have been widely exploited for analysis in physiological samples, e.g. the amperometric determination of PCT in blood and serum [204] or the use of voltammetric sensors based on chemically modified glassy carbon electrodes improving the heterogeneous charge-transfer rates and the selectivity of the working potentials [205,206].

The determination of the PCT in pharmaceutical samples has provided a large variety of procedures dealing with different kinds of detection: spectrophotometric and fluorimetric determinations and electrochemical methods involving ion-selective electrodes [207] and potential scanning voltammetric detection [208]. The reversible redox couples (Fe(III)/Fe(II); Ce(IV)/Ce(III); $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$; I_2/I^-; Br_2/Br^-; VO_3^-/VO^{2+}) were studied as indicating redox systems for biamperometric determination of paracetamol in a flow-injection assembly [209]. Considering the selectivity of assayed systems against excipients and antioxidants that typically are presented in pharmaceutical formulations (e.g. sucrose, glucose, ascorbic acid and hydrogen sulphite) the systems Fe(III)/Fe(II) and VO_3^-/VO^{2+} were selected for the determination of the PCT. The sample was injected into a carrier which merged with the oxidant stream where the drug was oxidised by excess oxidant.

Some papers describes enzyme-modified pre-column which can be coupled with amperometric or voltammetric techniques, showing inherent advantages as simplicity, low cost and rapidity. A continuous-flow sensing device with an enzyme-modified pre-cell coupled to an amperometric detector is proposed for determining PCT in pharmaceutical formulations, using a simple dissolution and filtration step before the measure [210]. Horseradish peroxidase (HRP) in the presence of H_2O_2 catalyses the oxidation of PCT to *N*-acetyl-*p*-benzoquinoneimine (NAPQI) whose electrochemical reduction back to PCT was obtained at peak potential of −0.10 V, proportionally to PCT concentration. Fatibello-Filho et al. [211] developed several biosensors and enzymatic batch and flow-injection procedures for determining various compounds using crude extracts or tissues of various vegetables instead of isolated enzymes. The use of such biological materials is very attractive because of their high stability, high enzyme activity concentration, very low cost and fewer cofactor requirements in comparison with the pure enzymes. In this way, a biosensor based on vaseline/graphite modified with avocado tissue (*Persea americana*) as the source of *polyphenol oxidase* was developed and used for the chronoamperometric determination of paracetamol in pharmaceutical formulations. This enzyme catalysis the oxidation of paracetamol to *N*-acetyl-*p*-benzoquinoneimine, whose electrochemical reduction back to paracetamol was obtained at a potential of −0.12 V.

A piezoelectric quartz crystal (PQC) has been selected as the transducer of the biosensor, because the sensor is very simple to assemble and has a fast response. In many cases the method requires no extraction, separation or concentration procedure and has the potential to be developed into a practical on-line method. For a PQC, however, there is no specific selectivity. As a result, various chemicals and biomaterials have been used to chemically or physically modify the PQC surface to obtain selectivity for a bulk acoustic wave (BAW) sensor. Pre-coated piezoelectric crystals have been successfully applied to biological, environmental and chemical fields. Unfortunately, many systems are either not as specific as expected for the chemicals, or not stable for the biomaterials. Therefore, it is necessary to seek stable, highly selective and facile materials as sensing components. One of these new techniques concerns molecularly imprinted polymers (MIPs).

Molecular imprinting technology is derived from the concept of creating designed recognition sites in macromolecular matrices by means of template polymerisation, by which artificial antibodies, receptors and enzymes can be created. This technique is based on in situ co-polymerisation of cross-linkers and functional monomers that form complexes with template (imprinted) molecules prior to polymerisation. After removing the template molecules from the formed material, binding sites are left behind showing complementarily to the template in a subsequent rebinding experiment. The complementary interactions between the functional monomers and the templates can be either non-covalent or reversible covalent in nature. The imprinted polymers based on non-covalent interactions between print molecules and suitable monomers have been widely applied. Firstly, MIPs have been used for the preparation of the stationary phases for chromatographic and electrophoretic techniques, with ability to efficiently separate the enantiomeric forms of peptides and drugs. Secondly, the polymers have also been used for the quantification of drugs in real samples using competitive binding assays. Thirdly, an area of interest is the use of imprinted materials as the recognition elements in biomimetic sensors. Finally, a variety of approaches have been followed in order to prepare polymers with catalytic properties.

The functional monomer commonly used in making MIP is methacrylic acid (MAA). However, MAA shows strong non-specific interaction with the imprinted molecule and the hydrogen bond between MAA and the imprinted molecule will be destroyed in a polar solvent such as acetonitrile. In order to improve the selectivity of MIP, polymerisation using MAA and 2-vinylpyridine as the functional monomers has been employed. In this context, a MIP–BAW sensor for paracetamol has been designed with an imprinted polymer as a sensing material [212]. Three kinds of polymers have been synthesised with two different kind of functional monomers. Through comparison, the polymer, simultaneously using 4-vinylpyridine (4-VP) and MAA as the functional monomers has a higher selectivity and sensitivity. From the kinetic impedance analysis, there was no change in the viscoelasticity of the polymer coating during the detection.

Use of electrochemical methods in analysis attracted attention as an accurate, sensitive and cost-effective method of analysis in last decade. Since voltammetric techniques are more selective, less costly and less time-consuming, they are widely used for the determination of PCT in pharmaceutical preparations. The electroanalytical features and performances of carbon paste

electrodes for fabrication of chemo and biosensors through the modification of carbon paste and their analytical applications are well documented. In last few years, the modification of electrode surface attracted considerable attention because of the remarkably improved results achieved in such cases. Bi et al. described a relatively simple and rapid electrochemical method by cyclic voltammetry using glassy carbon electrode for the detection of PCT in 1.0 M HCl solution [213]. Voltammetric determination of PCT at chemically modified electrodes [214,215], boron doped diamond film electrode [216] and at other electrodes [217,218] have also attracted attention, however, the lowest detection limit of 1.2 μM is reported at nafion/ruthenium oxide pyrochlore chemically modified electrode.

Owing to their novel optical, electronic, magnetic and catalytic properties gold nanoparticles are one of the most intensively studied and one of the most popular materials to be assembled on electrodes. It has been reported that the small size of gold nanoparticles allow the conductive materials to come into the vicinity of the active process providing bioelectrocatalytic activity that can be utilised in the construction of biosensors. Gold nanoparticles-modified electrodes are used increasingly in many electrochemical applications since they have the ability to enhance the electrode conductivity and facilitate the electron transfer, thus, improving the analytical selectivity and sensitivity. Normally peculiar binding molecules are used to assemble gold nanoparticles on the electrode surfaces but this may alter the conducting properties of the modified electrode. Recently, Oyama and co-workers have presented a new method to fabricate a gold nanoparticles-attached indium tin oxide (Au/ITO) electrode without using peculiar binding molecules, and this electrode was used for the determination of paracetamol at pH 7.2 [219]. The electrode exhibited an effective catalytic response to the oxidation of paracetamol with good reproducibility and stability.

A simple differential pulse voltammetric technique, for the sensitive and selective determination of PCT, at C_{60}-modified glassy carbon electrode at physiological pH, i.e. at pH 7.2, is described [220]. The electrode has a catalytic function towards the oxidation of PCT. Electrode modified using fullerene films have been found to show much better results as compared to bare electrodes and have a lot of potential applications in electroanalytical studies. The method described overcomes the two major limitations of conventional methods for the screening of PCT and its metabolites in urine samples, namely the hydrolysis time and the interference. Further, the method does not require any sample pre-treatment. The proposed method also resolves the voltammetric response of the paracetamol and *p*-aminophenol in two well-defined voltammetric peaks.

Series dual-electrode detectors with electrodes oriented according to the flow axis have become a well-established technique for liquid chromatography, capillary electrophoresis and flow-injection analysis and some advantages of this kind of cell design have been reported in literature. This approach has also been used for the on-line quantitative removal of electroactive interfering species and in titrations where the reactant is produced electrochemically, the end-point being detected amperometrically at the other electrode by monitoring the current appearance. Recently, it developed a thin-layered dual-band electrochemical cell with an interelectrode gap of 0.1 mm which operated at flowing conditions. Studies involving the performance of the dual-band channel electrode at different cell geometries by varying the channel height and the gap width between both electrodes were performed. Electrodes with generator–collector properties can be successfully used to study electrochemical reaction mechanisms, as reported in the pioneering studies of Anderson and Reilley where two working electrodes (twin-electrode) closely separated in a thin-layer cell configuration were used. Besides the well-known rotating ring-disc electrode, new strategies to fabricate generator–collector systems have appeared in literature and most of them are based on the well-known features associated with electrodes with at least one dimension in the range of micrometers. Interdigitated microelectrodes, ring-disc microelectrodes, closely spaced microelectrodes, band microelectrodes and a micromachined wall-jet ring-disc electrode are typical examples. A different approach involves a microdisc electrode and a larger counter electrode arranged face to face at a distance which can be precisely adjusted by means of a positioning mechanical system. Literature has reported the possibility of using recordable CDs to fabricate gold microelectrodes in single, twin or arrays configurations. In this context, it is extending this application in an attempt to construct twin-electrodes in thin-layer electrochemical cells. In this case, the species generated at one of the electrodes diffuses in opposite direction across the thin layer and is collected at the adjacent one, polarised to a level at which a reverse reaction can occur. A distinguished characteristic of twin-electrodes compared to RRDE is that, the collected species is able to diffuse back to the generator electrode and this redox cycling enhances the currents of both generator and collector electrodes (feedback effect). The fabrication of a new electrochemical cell where a thin layer of solution is confined between two closely spaced parallel gold electrodes is described [221]. By polarising both electrodes at suitable potentials, the forced redox cycling ensures that steady-state conditions are attained almost instantaneously. Voltammetry with ferricyanide solutions demonstrated that the twin-electrode thin-layer cell (TETLC) may operate as a generator–collector device with collection efficiency values of 100%. The increased sensitivity based on the forced redox cycling envisages future applications of the device, as the use of the TETLC as a pre-reactor at quiescent solutions for the determination of paracetamol in solutions containing ascorbic acid.

The combination of FIA with electrochemical detection is attractive because of the flexibility of the former and the diagnostic power of the latter. Two classes of electrochemical measurement are employed in flow detection: one class is based on charge transfer between a liquid or gaseous phase containing the analytes and a solid or immiscible liquid phase that is electrically conductive or semiconductive, and includes the most common potentiometric, voltammetric and coulometric detection techniques, and the other class involves the measurement of the electrical properties of liquids, i.e. the electrical conductivity and relative permissivity. There are many designs of electrochemical flow-through cell mainly those for continuous, on-line

monitoring and chromatographic application. The commercial electrochemical flow-through cells that widely used are very expensive; a low cost home-made electrochemical flow-through detector cell is fabricated to use in conjunction with a FI system with voltammetric detection [222]. The development of an extremely inexpensive electrode based on the use of pencil lead (graphite) is also described. The performance of the home-made electrochemical flow-through cell is evaluated by comparison with that of the commercial one by mechanistic study of PCT oxidation using cyclic voltammetry. The home-made cell is easy to fabricate and use. It is made from cheap and easily available materials which enable the home-made cell to be far cheaper than the commercial one. In addition, the stability of the pencil lead electrode used in conjunction with the designed flow-through cell gives rise to accurate and reproducible results which are as reliable as those obtained by using the commercial cell with the glassy carbon electrode.

Oscillating reactions are complex dynamic systems that involve cyclic or periodic changes in the concentration of some ingredient (whether a reactant, a product or an intermediate) with time. Oscillating reactions have so far been analysed mainly in physical–chemical terms in order to elucidate the complex mechanism that they involve. Two of the better known oscillating chemical systems are the Belousov–Zhabotinskii (BZ) reaction and the Bray–Liebhafsky reaction. From available knowledge on these reactions, it follows that the system must be far from thermodynamic equilibrium, which entails using a well stirred continuous-flow reactor (CSTR), and that one or more autocatalytic or cross-catalytic steps must take place between two steps of the reaction mechanism. Although some oscillating reactions have been used for analytical purposes, particularly the Ce(IV)-catalysed reaction between malonic acid and $KBrO_3$ (BZ reaction), analytical determinations based on a closed system including the analyte involve labour-intensive procedures that entail re-starting the oscillating system before each new determination. The recent inception of an open system for application of the analyte pulse perturbation (APP) technique by use of a CSTR has opened up new avenues for oscillating reactions in routine analyses. As a result, there has been a gradual shift from theoretical to practical interest. The APP technique allows a system to be kept oscillating for a long time and thus be employed as an inexhaustible indicator system for successively added samples and/or standards. This technique is a useful analytical tool inasmuch as it uses very simple, modular equipment that can be assembled from parts available in any analytical laboratory. The application of the oscillating reaction-based determinations to the analysis of real samples was addressed by use of the APP technique. The oscillating reaction of the H_2O_2–NaSCN–$CuSO_4$ system in an alkaline medium was chosen for this purpose. The addition of small amounts of vanillin, paracetamol and ascorbic acid to the oscillating system alters some of its properties; this effect can be used to develop methods for the determination of the analytes in real samples [223]. For paracetamol, second period method, or total-period method was correlated with the amount of analyte added; calibration curve provided by first method was linear, whereas that obtained with the total-period method fitted a second-order polynomial.

Pejic et al. [224] develop a method based on the pulse perturbation of the oscillatory reaction system being in a stable steady state (PPOSSS) in the vicinity of a bifurcation point, for quantitative determination of the paracetamol using Bray–Liebhafsky (BL) oscillatory reaction as the matrix and, in particular, to demonstrate that the mentioned kinetic method can be successfully applied for quantitative determination of paracetamol in bulk drug and pharmaceutical preparations. By perturbing the matrix system being in a stable steady state, it is not necessary to test oscillatory phases and to perturb the system always in the same selected oscillatory phase point, which is very delicate moment. Comparing with the matrix system being in the oscillatory state, the regeneration of the system being in the stable non-equilibrium stationary state (stable steady state) is shorter. The method is based on potentiometric monitoring of the concentration perturbations of the matrix reaction system being in a stable non-equilibrium stationary state close to the bifurcation point. The response of the matrix system to the perturbations by different concentrations of paracetamol is followed by a Pt electrode.

Other electroanalytical determinations of PCT are showed in Table 4.

4. Chromatographic methods

4.1. Pharmaceutical preparations

The simultaneous determination of the active ingredients in multicomponent pharmaceutical products normally requires the use of a separation technique, such as gas chromatography or high performance liquid chromatography, followed by quantitation.

4.1.1. High performance liquid chromatography

Among the various analytical techniques, HPLC constitutes the most popular chromatographic method for separating mixtures of analgesic drugs and related compounds. For reliable quality control of analgesic products, a selective and versatile detection system is desirable to aid in the positive identification of the column effluents. To this end, diode-array detection (DAD) was used in combination with postcolumn on-line photochemical derivatisation. Using a photoreactor arranged on-line between the column and the DAD instrument, the column effluent was subjected to UV irradiation (254 nm) and photo-induced chromophore alterations resulted in modified UV spectral properties of the analytes. Thus, the known efficacy of the DAD in confirming the peak identity was enhanced, enabling two different UV spectra (photoreactor on and off) to be obtained for each analyte.

HPLC analyses of pharmaceutical dosage forms containing analgesics and related compounds (acetylsalicyclic acid, paracetamol, propyphenazone, caffeine and chlorpheniramine) were performed on C_{18} and cyano columns under reversed-phase conditions [237]. The performance of the methods was enhanced by introducing postcolumn on-line photochemical derivatisation in combination with diode-array detection. The column effluents were subjected on-line to UV irradiation (254 nm) and the char-

Table 4
Electroanalytical methods for the determination of paracetamol

Sample type	Method	Remark	Reference
Biological fluids (blood serum and urine)	a.c. polarography	After elimination of protein by perchloric acid, followed by reaction with nitrous acid in acidic medium, the nitro derivative is measured at pH 10	[225]
Pharmaceutical dosage form	Polarography	Paracetamol and salicylamide after treatment with nitrous acid	[226]
Blood plasma (rat serum) and tablets	Differential pulse voltammetry	Oxidation of PCT on a carbon paste electrode	[227]
Urine and pharmaceutical preparations	Differential pulse voltammetry	Using a pumice mixed carbon paste electrode	[228]
Pharmaceutical products	Cyclic voltammetry	Using crude extracts of several fruits and vegetables as the sources of peroxidise for use in carbon paste biosensors	[229]
Pharmaceutical products	Differential pulse voltammetry	Simultaneous determination of paracetamol, ascorbic acid and caffeine; using a glassy carbon electrode	[230]
Pharmaceutical products	Differential pulse stripping voltammetry	Paracetamol and phenobarbital; using a glassy carbon electrode; assisted by chemometrics (PLS and PCR methods)	[231]
Plasma	Amperometric	Electrochemical oxidation of PCT; the technique has been adapted for assay in biological fluids by the use of a permselective cellulose acetate membrane, and an outer diffusion-limiting, microporous polycarbonate membrane treated with dimethyldichlorosilane to impart biocompatibility	[232]
Tablets	Flow-injection analysis with biamperometric detection	Oxidation with Ce^{4+} in acidic medium; dual-channel flow-injection system incorporating an amperometric detector with two Pt microelectrodes	[233]
–	Coulometric titration	With electrogenerated bromide; the monobromination of PCT is fast during which the concentration of electrogenerated bromide is low that methyl orange indicator is not bleached until after monobromation	[234]
Combined dosage forms	Potentiometric titration	Paracetamol and mefenamic acid; using orthogonal polynomial coefficient ratio	[235]
Pharmaceutical preparations	Potentiobromometric technique	Oxidation with bromide in acetate buffer media; using Ag amalgam electrode or bromide ion-selective electrode	[236]

acteristic photo-induced spectral modifications were useful for the unambiguous identification of the various analgesic compounds.

An isocratic HPLC method for the simultaneous determination of the active compounds paracetamol, pseudoephedrine and chlorpheniramine in chewing gum samples has been developed by Gasco-Lopez et al. [238]. The method required a simple liquid–liquid extraction using *n*-hexane and a mixture of water–acetonitrile prior to HPLC analysis. The chromatographic separation was achieved with an aqueous solution containing hexylamine (pH 3)–acetonitrile as the mobile phase, a Spherisorb C_{18} column and UV detection at 220 nm.

Since instrumental planar chromatography is regarded as a reliable, fast and accurate method for quantitative drug analysis, it is proved HPTLC as a technique which can be used as an alternative method of drug assay. A simple and rapid HPTLC method for the determination of acetylsalicylic acid, paracetamol, caffeine and phenobarbitone in dosage form is presented [239]. The determination of analgoantipyretics were performed on pre-coated HPTLC silica gel plates (10 cm × 20 cm) by development in the mobile phase dichlormethane:ethyl acetate:cyclohexane:isopropanol:0.1 M HCl:formic acid (9:8:3:1.5:0.2:0.2, v/v/v/v/v/v). TLC scanner was used for direct evaluation of the chromatograms in the reflectance/absorbance mode. Other paper describes a RP-HPLC method with UV detection without prior derivatisation for direct simultaneous determination of acetylsalicylic acid, caffeine, paracetamol and phenobarbital in tablet form. A compromise wavelength for compounds investigated was 207 nm, found to be satisfactory for use of the proposed method in the routine control of multidrug pharmaceutical preparation, using chromatographic system consisting a Bio Rad 18 01 solvent pump, Rheodine 71 25 injector and Bio Rad 18 01 UV–Vis Detector. Separation was achieved using Bio SiL HL C_{18}, 5 μm, 250 mm × 4.6 mm column. Mixture of acetonitrile:water (25:75, v/v) adjusted to pH 2.5 with phosphoric acid was used as a mobile phase at a flow rate of 2.0 ml min^{-1} [240].

Paracetamol, caffeine and codeine phosphate were separated using a μBondapack C_8 column by isocratic elution with flow rate 1.0 ml min^{-1}. The mobile phase composition was 420:20:30:30 (v/v/v/v) 0.01 M KH_2PO_4, methanol, acetonitrile, isopropyl alcohol and spectrophotometric detection was carried out at 215 nm [241].

Under abnormal conditions (heat, pH, temperature, etc.), PCT degrades slowly forming a mixture of contaminants, such as 4-aminophenol and acetic acid. This reaction could also be carried out by enzymatic cleavage or by microwave assisted alkaline hydrolysis. In addition to 4-aminophenol, 4-chloroacetanilide

could also be present as impurities in the starting material of paracetamol in which it should be controlled. Therefore, it is very important to have an analytical technique for the measurement of PCT and its degradation product simply and precisely. A variety of high performance liquid chromatographic methods have been proposed for such measurement, using silica-based stationary phases [242–244]. These methods are time consuming in addition, the alkyl bonded silica-based stationary phases suffer from a number of drawbacks, including poor stability at extremes of pH and a variety of unwanted interactions due to surface heterogeneity. However, due to the instability of these packing materials and the complicated separation systems, porous graphitised carbon (PGC) showed to be preferable packing for the separation and determination of drug and pharmaceuticals. This material has several advantages, most notably its physical and chemical stability, as well as superior selectivity towards diasteriomers and geometric isomers and also is most applicable to the separation of small ionisable molecules that are not retained with octadecylsiloxane (ODS) columns. Monser and Darghouth [245] describe a simple and rapid HPLC method for simultaneous determination of paracetamol, 4-aminophenol and 4-chloroacetanilide using PGC column. Further, studies were carried out on the effect of the mobile phase composition and pH in the retention of these compounds on PGC column. The chromatographic separation was achieved on porous graphitised carbon column using an isocratic mixture of 80:20 (v/v) acetonitrile/0.05 M potassium phosphate buffer (pH 5.5) and ultraviolet detection at 244 nm.

4.1.2. Planar chromatography (thin-layer chromatography, TLC)

TLC is a technique related to HPLC but with its own specificity. Although these two techniques are different experimentally, the principle of separation and the nature of the phases are the same. Due to the reproducibility of the films and the concentration measurements, TLC is now a quantitative method of analysis that can be conducted on actual instruments. The development of automatic applicators and densitometers has lead to nano-TLC, simple to use technique with a high capacity.

A thin-layer chromatography–UV scanning densitometric technique is used for the simultaneous determination of paracetamol and chlorzoxazone [246]. A favourable advantage of TLC–UV densitometry over UV spectrophotometry is its ability to separate the contents of the analysed samples, thus eliminating the possibility of interference between active ingredients or due to additives, excipients or impurities. In addition, the method is amenable to the simultaneous analysis of six samples on the same TLC plate with precision and accuracy comparable with alternate UV and derivative spectrophotometry. Other advantages of the TLC–UV method are its fast scanning speed, its low limit of detection and its broad linear ranges.

4.1.3. Micellar liquid chromatography (MLC)

MLC is a mode of reversed-phase liquid chromatography, which uses aqueous solutions of surfactants above the critical micellar concentration. This chromatographic system presents some differences with respect to the classical reversed-phase chromatography because the stationary phase is modified by the absorption of surfactant and the mobile phase presents surfactant micelles. This system provides hydrophobic, electronic and steric sites of interaction for solutes that allows the effective separation of compounds of different nature. In addition, the solubilisation capability of the micellar solutions simplifies the sample preparation step and reduces the consumption of organic solvents.

Rapid chromatographic procedures for analytical quality control of pharmaceutical preparations containing antihistamine drugs, alone or together with other kind of compounds are proposed. The method uses C_{18} stationary phases and micellar mobile phases of cetyltrimethylammonium bromide (CTAB) with either 1-propanol or 1-butanol as organic modifier. The proposed procedures allow the determination of the antihistamines: brompheniramine, chlorcyclizine, chlorpheniramine, diphenhydramine, doxylamine, flunarizine, hydroxyzine, promethazine, terfenadine, tripelennamine and triprolidine, in addition to caffeine, dextromethorphan, guaifenesin, paracetamol and pyridoxine in different pharmaceutical presentations (tablets, capsules, suppositories, syrups and ointments) [247].

4.1.4. Sequential injection chromatography (SIC)

Sequential injection analysis, developed by Ruzicka and Marshall in 1990, represents advanced form of solution manipulation available to analytical chemists for mixing and transport of samples, reagents and products of chemical reactions to the measurement point. Fast and intensive development of SIA methodology was due to several factors essential for routine analytical determinations, e.g. simplicity of fundamental principles, inexpensive instrumentation, automated sampling and analytical procedures, limited sample consumption, short analysis time, on-line performance of difficult operations (preconcentration, physical–chemical conversion of analytes into detectable species, dialysis, stopped-flow technique, etc.).

Sequential injection chromatography is a new area of analysis within the SIA. A simple SIC system could be developed by incorporating reversed-phase monolithic columns into the commercially available SIA manifold. Monolithic supports have become the subject of extensive study in recent years and they were developed on the basis of a new sol gel process, which includes the hydrolysis and polycondensation of alkoxysilanes. In contrast to conventional HPLC columns, monolithic columns are formed from a single piece of porous silica gel (monolith). The performance of the reversed-phase monoliths is equivalent to a typical C_{18} 5 μm particulate HPLC column. Due to the presence of large through-pores and mesopores, the monoliths possess a much higher porosity than conventional particulate HPLC columns. The resulting column back-pressure is therefore much lower and allows operation in a SIA system. Thus, it can be incorporated into an SIA system regardless of the limitations of the syringe pump.

A new separation method for simultaneous determination of paracetamol, caffeine, acetylsalicylic acid and internal standard benzoic acid was developed based on a novel reversed-phase sequential injection chromatography technique with UV detection [248]. A Chromolith Flash RP-18e, 25–4.6 mm column and

a FIAlab 3000 system with an eight-port selection valve and a 5 mL syringe were used for sequential injection chromatographic separations in this study. The mobile phase used was acetonitrile–0.01 M phosphate buffer (10:90, v/v) pH 4.05, flow rate 0.6 mL min^{-1}. UV detection was at 210 and 230 nm.

Other proposed chromatographic methods for the determination of PCT are shown in Table 5.

4.2. Biological fluids

PCT is used extensively in the treatment of mild to moderate pain and fever. It has been used in the treatment of pain in combination with aspirin, caffeine, opiates and/or other agents. PCT–opiates combination produces greater analgesic effect than the produced by either acetaminophen or higher doses of the opiate alone. Treatment of pain often requires parenteral administration of analgesics. However, acetaminophen is not available for parenteral use. Contrary to the latter propacetamol HCl can be easily solubilised and is suitable for parenteral administration. Propacetamol, the diethylamino-acetic ester of acetaminophen is a pro-drug that is very quickly and quantitatively hydrolysed into acetaminophen by plasma esterases within 7 min after intravenous injection.

The preferred analytical method to emergency estimation of the plasma acetaminophen concentration is HPLC. Several assays for acetaminophen using HPLC are suitable for analysis in pharmaceutical preparations, and others are suitable for analysis in biological fluids at therapeutic to toxic concentrations of the drugs (Table 3). Such methods are, however, not enough sensitive when sub-therapeutic drug levels must be measured as frequently occurs in pharmacokinetic studies. Methods reporting the sensitivity necessary to quantify pharmacokinetics acetaminophen levels accurately have also been published [280–282]. Most of these methods used HPLC in conjunction with electrochemical detection, solid-phase extraction and sample extraction procedures that involve multiple time-consuming organic extractions and solvent evaporation steps [282–285]. Only a few of these methods [280,282,286] are available for rapid measuring the large number of samples that have to be analysed in a pharmacokinetic study. All of these procedures require at least 1 ml of plasma. Consistently, pharmacokinetic studies after acetaminophen–drug combinations have not been completed, as plasma samples of 3–4 ml cannot be repeatedly obtained from human subjects. One method [280] is available for measuring acetaminophen at toxic and pharmacokinetic levels. Plasma concentrations of acetaminophen above 100 μg ml^{-1} at 4 h and 50 μg ml^{-1} at 12 h after drug ingestion cause hepatotoxicity. Acetaminophen plasma concentrations below 0.1 μg ml^{-1} were found 12 h after 325 mg oral doses of acetaminophen.

For this reason, a simple method for the rapid estimation of acetaminophen in plasma is investigated and described [287]. *p*-Propionamidophenol was used as internal standard. The assay involved a single ethyl acetate extraction and LC analysis at a wavelength of 242 nm using a reversed-phase encapped column, with a mobile phase of acetonitrile and 0.005 M potassium dihydrogen phosphate adjusted at pH 3.00. The limit of quantitation of acetaminophen by this method was 0.05 μg ml^{-1}, only 0.1 ml of the plasma sample was required for the determination. Analytical methods have been developed that estimate the concentrations of the major metabolites of paracetamol by hydrolysing the paracetamol glucuronide (PG) and paracetamol sulphate (PS) back to paracetamol, or reporting concentrations of the metabolites as paracetamol equivalents [286,288]. Other assays quantify the major metabolites but they involve relatively complex liquid–liquid extraction techniques [289], or lack the sensitivity required for single-dose metabolism studies in humans [286,290], or gradient chromatography [291]. While some assays report adequate sensitivity they lack the accuracy and precision required in single-dose studies, and therefore have not been applied [292]. Other document reports the successful development and validation of a simple, selective and sensitive isocratic HPLC method for the simultaneous quantification of paracetamol in plasma and of PG and PS in both plasma and urine [292]. A distinct advantage of this method is that it requires minimal sample volume (<100 μl) allowing finger-prick blood sampling during pharmacokinetic evaluation studies.

Aspirin is no longer recommended as an analgesic or antipyretic for children and neonates because of reported associations with Reye's syndrome. As a result, PCT is now the standard analgesic/antipyretic for such patients. However, it is well known that PCT, which is extensively metabolised by the liver mainly by conjugation to the sulphate and glucuronide, may be converted to a highly toxic oxidative metabolite, *N*-acetyl-*p*-benzoquinoneimine (NABQI) when administered in overdose. The metabolite, which is formed through the intermediary of cytochrome P450 mixed function oxidase, is usually detoxified by combining with glutathione. However, when PCT is administered in overdose, glutathione stores are depleted and toxicity ensues. Therefore, it is important to know how children with chronic liver disease metabolise the drug. Young children and neonates excrete more of an oral dose of paracetamol as the sulphate than do adults. Indeed, in the young (<12 years), conjugation to the sulphate is the predominant elimination pathway, while glucuronidation is more important in older subjects. As part of a study to investigate the safety of paracetamol in children with liver disease, a HPLC method was developed for assaying paracetamol and its metabolites in body fluids [290]. Moreover, the possible use of salivary samples for defining the drug's pharmacokinetics in such children was evaluated. The method for quantifying the metabolism of paracetamol in children with chronic liver disease and the good correlation between plasma and salivary concentrations of paracetamol is demonstrated. Despite an increasing bias between the two methods with increasing concentration of paracetamol, it is concluded that salivary assay is satisfactory for characterising paracetamol pharmacokinetics in the group of patients studied.

Despite its common use, pharmacokinetic data about paracetamol is scarce, especially in young infants and neonates. Due to differences in their metabolism, there is special interest in the levels of PCT in neonates, particularly after multiple dosing. In neonates, the volume of blood taken is limited by ethical considerations and thus a method requiring only small volumes of blood is desirable. The use of a collection card similar to the Guthrie paper card used for the sampling of small volumes

Table 5
Determination of paracetamol in formulations by chromatographic methods

Other components	Method	Remark	Reference
–	Reversed-phase HPLC	Using a C_{18} stationary phase with methanol–water (1:2, v/v) mixture at the flow rate of 1.78 ml min^{-1} with detection at 193.3 nm; sulphamethoxazole is used as an internal standard	[249]
Acetylsalicylic acid	Stability-indicating GLC	In suppositories; samples were dissolved in chloroform and absolute alcohol (4:1) and gentisic acid as internal standard; solvents evaporated and silylation accomplished by adding BSA and BSTFA (1:1); using a flame ionisation detector; column of 2% OV-225 on Chromosorb W; nitrogen used as carrier	[250]
Chlormezanone	Reversed-phase HPLC	On a INERTSIL ODS-3V column at 228 nm	[251]
Chlorzoxazone	HPLC	Using salicylic acid as an internal standard	[252]
Chlormezanone Chlorzoxazone	GLC	In single and combined dosage forms; using 10% OV-17 column; flame ionisation detector; phenacetin as internal standard	[253]
Ibuprofen	GLC	Using on column derivatisation technique	[254]
Mefenamic acid	HPTLC	On silica gel $60F_{254}$ HPTLC plates with toluene–acetone–methanol (8:1:1, v/v/v) as mobile phase; detection at 263 nm	[255]
Acetylsalicylic acid, ascorbic acid	Reversed-phase HPLC	Using a C_8 column; mobile phase was methanol–0.2 M phosphate buffer (pH 3.5)–water (20:10:70)	[256]
Acetylsalicylic acid, ascorbic acid	HPLC	Simultaneous determination	[257]
Butalbital, caffeine	Micellar electrokinetic chromatography (MEKC)	Uncoated fused silica capillary (70 cm × 50 μm i.d., 50 cm to detector) was used for the separation at an applied voltage of 20 kV. A phosphate run buffer (pH 9, 0.05 M) containing 0.05 M sodium dodecyl sulphate was used for analysis. Separations of the analytes were achieved within 12 min at ambient temperature with detection set at 220 nm	[258]
Caffeine, dipyrone	HPLC	Using a μ-Bondapack C_8 column by isocratic elution with a flow rate of 1.0 ml min^{-1}; mobile phase was 0.01 M KH_2PO_4–methanol–acetonitrile–isopropyl alcohol (420:20:30:30); detection at 215 nm	[259]
Caffeine, propyphenazone	Reversed-phase HPLC	Mobile phase is a linear water–methanol gradient	[260]
Codeine phosphate, sodium benzoate	Reversed-phase HPLC	Using a stability-indicating method; in an elixir formulation; the reverse-phase paired-ion method utilises UV detection at 214 nm, a C_{18} column at 50 °C	[261]
Chlorzoxazone, diclofenac sodium	GC, HPLC and HPTLC	The results obtained by the different techniques are comparable	[262]
Chlorzoxazone, diclofenac sodium	Reversed-phase HPLC	Using ketoprofen as internal standard; on Zorbax C_8 column using a mixture of acetonitrile: 50 mM disodium hydrogen orthophosphate; at 220 nm	[263]
Chlorzoxazone, ibuprofen	GC, HPLC and HPTLC	The results obtained by the different techniques are comparable	[264]
Chlorzoxazone, ibuprofen	Reversed-phase HPLC	On Kromasil C_8 column using a mixture of 0.2% triethylamine: acetonitrile; at 215 nm; using ketoprofen as internal standard	[265]
Chlorzoxazone, oxyphenbutazone	Reversed-phase HPLC	–	[266]
Diclofenac sodium, methocarbamol	HPLC and SFC	Using packed column SFC employing internal standard method. The analytes were resolved by elution with supercritical fluid carbon dioxide doped with 11.1% (v/v) methanol on a Shendon-Phenyl (250 mm × 4.6 mm) 5 μm column with detection monitored spectrophotometrically at 225 nm. SFC method was compared to an HPLC assay	[267]
Ibuprofen, methocarbamol	Reversed-phase HPLC	A Bondapak(TM) phenyl column 30 cm from waters in isocratic mode, with mobile phase 0.2% orthophosphoric acid and methanol (45:55, v/v); at 215 nm	[268]
Phenylephrine HCl, triprolidine HCl	HPLC and HPTLC	The results obtained by the different techniques are comparable	[269]
Pseudoephedrine HCl, triprolidine HCl	Reversed-phase HPLC	Isocratic; a wavelength switching programme to the use of a compromise wavelength for all three compounds	[270]
Acetylsalicylic acid, caffeine, D-propoxyphene hydrochloride	HPLC	–	[271]
Acetylsalicylic acid, caffeine, guaiacol glycerol ether, propyphenazone	Thin-layer chromatography	Densitometric determination; using an external standard for calibration; adsorption measurement (reflectance detection) in situ	[272]
Acetylsalicylic acid, ascorbic acid, caffeine, salicylic acid	Chromatography	Densitometric determination in UV at 280 nm	[273]

Table 5 (*Continued*)

Other components	Method	Remark	Reference
Caffeine, Chlorpheniramine maleate, glycerylguaiacolate, phenylpropanolamine hydrochloride	HPLC	With diode-array detection	[274]
Ascorbic acid, caffeine, chlorpheniramine maleate, dextromethorphan HBr monohydrate	Mixed ion-pair HPLC	Dual-wavelength detection; using tetrabutylammonium hydrogen sulphate and pentane sulphonic acid	[275]
Acetylsalicylic acid, caffeine, codeine, pyridoxine, thiamine	Reversed-phase HPLC	Using a C_{18} Nucleosil column; mobile phase consist of two successive eluents: water (5 min) and acetonitrile–water (75:25, v/v, 9 min)	[276]
Caffeine, codeine, phenacetin, phenyltoloxamine, salicylamide	Reversed-phase HPLC	A 10 μm C_{18} silica gel stationary phase is used with a methanol–acetonitrile–water–tetrahydrofuran mixture (20:20:55:5, v/v/v/v); at 254 nm	[277]
Guaifenesin, pholcodine, pseudoephedrine, together with a series of parabens preservatives	HPLC	Using a chemically bonded octadecylsilane stationary phase with a mobile phase of methanol–water–acetic acid (45:55:2) containing the ion-pairing agent octanesulphonic acid	[278]
Forty analgesic drugs	HPLC	On an ODS-silica packing material; using three isocratic eluents prepared from isopropanol, formic acid and aqueous phosphate buffer	[279]

of blood allows more frequent sampling while still complying with ethical guidelines of a maximum 1 ml kg^{-1} body weight for blood sampling from neonates. The use of blood spot collection cards is a simple way to obtain specimens for analysis of drugs with a narrow therapeutic window. Oliveira et al. [291] describe the development and validation of a microanalytical technique for the determination of PCT and its glucuronide and sulphate metabolites from blood spots. The method is based on reversed-phase high performance liquid chromatography with UV detection. The small volume of blood required (20 μl), combined with the simplicity of the analytical technique makes this useful procedure for monitoring paracetamol concentrations.

The use of marker compounds for estimating drug metabolic capacity or pharmacokinetic parameters is common in the biological sciences. Often small laboratory animals are used and thus sample size is a limiting concern. Other study describes an assay for measuring the concentration of acetaminophen and its conjugated metabolites in low volume serum samples [293]. Acetaminophen and metabolites were removed from 10 μl serum samples by a single-step 6% $HClO_4$ deproteination using theophylline as internal standard. Samples were separated in a pH 2.2 sodium sulphate:acetonitrile mobile phase at a flow rate of 1.5 ml min^{-1} on a 15 cm octadecylsilyl column at room temperature. Analytes were detected at a wavelength of 254 nm.

Many HPLC methods have been reported for the determination of PCT and ceterizine (CTZH) hydrochloride in biological fluids, or in pharmaceutical formulations. But, none of these methods demonstrate the simultaneous quantification of these two drugs in combination in human plasma and pharmaceutical preparations. Moreover, the reported methods are not satisfactory for routine quality assurance for one or the other reason. Some of these methods have low sensitivity or works out only at higher concentrations of the drugs or need more time for analysis. Hence, it was felt necessary to develop a simple and sensitive HPLC method, which does not suffer from the above limitations for the determination of binary combination of CTZH and PCT in human plasma and pharmaceutical formulations. An accurate, simple, reproducible and sensitive HPLC method for the determination of CTZH and PCT has been developed and validated [294]. The separation of CTZH, PCT and Nimesulide (the internal standard) was achieved on a CLC C_{18} (5 μm, 25 cm × 4.6 mm i.d.) column using UV detection at 230 nm. The mobile phase was consisted of acetonitrile–water (55:45, v/v).

On the other hand, the practice of clinical and forensic toxicology involves among other things, the screening of acidic and neutral drugs in physiological fluids. Among the drugs of interest are analgesics (including paracetamol and salicylic acid), anticoagulants, antidiabetics, antiepileptics, barbiturates, diuretics, hypnotics, muscle relaxants and xanthine derivatives. The widespread use of PCT to counter a variety of ailments, such as pains, insomnia, hypertension, etc., may have contributed towards the high incidents of overdosage with these drugs. Of the published analytical methods on acidic and neutral drugs screen, reversed-phase HPLC–DAD offers a number of advantages. As most of the drugs of interest are readily eluted by the solvent systems used and are amenable to UV detection, the HPLC–DAD method offers one of the most comprehensive screens for the presence of these drugs. As UV response is usually proportional to the concentration of a drug, the method provides quantitative assay of these drugs over a wide concentration range from sub-therapeutic to fatal levels. Furthermore, the full UV/vis spectrum of a drug obtained by DAD provides a simultaneous and yet independent confirmation of the identity of the drug. These salient features make the HPLC–DAD method one of the most desirable methods for the screening of acidic and neutral drugs in blood. The HPLC–DAD method no matter how perfect is not without shortcomings. Among the drugs of interest, some are not amenable to UV detection while a number

of others are not amenable to DAD confirmation because of lack of UV absorption maxima and minima. A GC method with flame ionisation detection (GC–FID) was instituted to complement the HPLC–DAD method [295].

Blood previously acidified with aqueous saturated ammonium chloride solution was extracted with ethyl acetate. The dried extract was subjected to acetonitrile–hexane partition. The acetonitrile portion was analysed for the presence of acidic and neutral drugs by HPLC–DAD (200 mm × 2.1 mm i.d. microbore ODS-Hypersil column) and GC–FID (25 m narrowbore × 0.25 mm i.d. HP-5 column with 0.33 μm film thickness). The protocol was found to be suitable for both clinical toxicology (including emergency toxicology) and postmortem toxicology. At least 66 drugs of interest were unequivocally identified by RRTs (HPLC) and UV spectra (DAD) match while another 12 were unequivocally identified by double RRTs match (HPLC and GC). Quantitation was facilitated by incorporating calibration blood standards in each assay batch.

An analytical method for the determination of paracetamol and chlorpheniramine in human plasma has been developed, validated and applied to the analysis of samples [296]. The analytical method consists in the extraction of paracetamol and chlorpheniramine with diethyl ether, followed by the determination of both drugs by a liquid chromatography–tandem mass spectrometry (LC–MS–MS) method, using 2-acetamidophenol as internal standard.

The purpose of other interesting study was to simplify, to speed-up and to assay simultaneously paracetamol and guaifenesin in human plasma using the LC–MS–MS technique which can be used for pharmacokinetic studies after oral administration of multicomponent formulations, containing paracetamol, guaifenesin, pseudoephedrine and dextrorphan [297]. After extracted from plasma samples by diethyl ether–dichloromethane (3:2, v/v), the analytes and internal standard osalmide were chromatographed on a C_{18} column. Detection was performed on a triple quadrupole tandem mass spectrometer by selected reaction monitoring (SRM) mode via atmospheric pressure chemical ionisation (APCI).

NMR spectroscopy is a powerful method for the analysis of endogenous and exogenous components in biological fluids. The main advantages of NMR spectroscopy for the analysis of biofluids are that; (i) NMR is a non-selective detector, monitoring the levels of all low molecular weight components in free solution above the detection threshold, (ii) the technique is fast and requires only low sample volumes, (iii) the information content of NMR spectra is very high, with, for instance, hundreds of human urine components being detected in ^{1}H NMR spectra of human urine, and most importantly and (iv) NMR spectra are inherently rich in structural information on each one of the components that are detected. For the structure elucidation of novel components in matrices as complex as human urine, it is extremely advantageous to couple a separation technology to the NMR, as has been achieved by LC–NMR. Improvements in NMR sensitivity and solvent suppression efficiency have made LC–NMR a viable and important tool for the identification of components in complex mixtures. The recent introduction of suppression methods which can be tailored to the width of the solvent peak allows the observation of signals close to the solvent, on-flow. However, to solve the molecular structure of a novel substance by NMR spectroscopy alone is often impossible. LC–MS is a well-established technology for determining the molecular weights of components in mixtures with excellent sensitivity but, as the ionisation process used for most LC–MS applications is relatively 'soft', little structural information is obtained. Coupled, on-line LC–NMR–MS is the logical combination of these two powerful and complementary analytical techniques. This technology can be implemented using commercially available equipment, paying particular attention to operation of the chromatograph and mass spectrometer within the stray field of the NMR magnet. The ionisation characteristics of biomolecules differ sufficiently that the mass spectrometer used in the system should be equipped to perform the full range of ionisation modes typically used in metabolism studies. Using LC–NMR–MS its can obtain UV, NMR and mass spectral data from components in mixtures as they elute from an HPLC in a single chromatographic run. This avoids the potential problem of mis-interpreting data from chromatographic components which change their elution order with small variations in HPLC conditions. The LC–NMR–MS technique gives far more structure elucidation power than available previously to solve the structures of components in complex mixtures rapidly and efficiently. Burton et al. [298] demonstrate the effectiveness of this new technology by its application to the analysis of the structures of the metabolites of PCT in human urine.

Finally, TLC remains a popular method for qualitative analysis and for the screening of samples such as urine for drugs. However, when compounds of interest are detected by this type of screening, confirmation of the identity of the compound(s) of interest is then usually undertaken using another method such as gas chromatography mass spectrometry (GC–MS). Very often this requires that the sample be subjected to extraction and subsequent derivatisation before the analyte is in a suitable form for chromatography. However, if the aim of the analysis is confirmation of identity, then the use of mass spectrometric techniques directly on the material present in the TLC spot/band represents a practical alternative to GC/MS, etc. In previous studies its have demonstrated the application of TLC/MS, and more recently TLC/tandem mass spectrometry (MS/MS) to a variety of compound types. This has included the use of TLC/MS and TLC/MS/MS for the identification of natural products, drugs and metabolite and industrial chemicals in a variety of matrices and from various TLC stationary phases. The results of studies using TLC/MS and TLC/MS/MS on non-steroidal anti-inflammatory drugs, analgesics and metabolites, both as pure substances and when present in urine extracts are presented [299]. TLC was combined successfully with mass spectrometry and with tandem mass spectrometry using silica gel and diol-bonded silica gel high performance TLC plates. The diol-bonded phase was found to be superior for use with biological samples and enabled the identification of paracetamol, ibuprofen and salicylhippuric acid (the major metabolite of acetylsalicylic acid) in human urine extracts following normal therapeutic doses.

Table 6
Determination of paracetamol in biological fluids by chromatographic methods

Other components	Method	Remark	Reference
–	HPLC	Ether extraction; on a 10 μm particle size silica gel column, using a mobile phase of 10% chloroform in terehydrofuran; in urine	[300]
–	Reversed-phase HPLC	With a single-step extraction; in plasma	[301]
–	HPLC	Applicable to single-dose pharmacokinetic studies; in plasma	[302]
–	HPLC	Improved PCT assay sensitivity by modification of HPLC	[303]
Its major metabolites	Micro-HPLC	In plasma and urine	[304]
Theophylline	HPLC	Extraction from postmortem tissues and urine	[305]
Its four major metabolites (glucuronide, sulphate, cysteine and mercapturate conjugates)	Reversed-phase HPLC	In mouse plasma samples; an ODS column was used and the mobile phase consisted of an aqueous solution of 0.01 M tetrabutylammonium chloride and 0.01 M tris buffered to pH 5.0 with phosphoric acid, with methanol as the organic solvent. The gradient elution started with 30% methanol. After a delay of 0.5 min the methanol concentration was increased linearly to 75% over 7.5 min. The column was returned to the initial conditions after a delay of 1 min	[306]
–	HPLC with dual-electrode coulometric quantitation	In blood and plasma; samples prepared by precipitation with trichloroacetic; in the redox mode (oxidation at +0.25 V followed by reduction at −0.15 V)	[307]
–	Micellar LC with a wall-jet cell/carbon fibre microelectrode	The separations were carried out in an analytical column packed with C_{18} stationary phase and the mobile phase was 0.05 mol l^{-1} sodium dodecyl sulphate containing 3% (v/v) *n*-propanol at pH 7.15; direct injection of urine	[308]
Its major metabolites	Reversed-phase HPLC	Employing UV detection (248 nm), 5 μm resolve C_{18} as stationary phase and 0.1 M potassium phosphate monobasic/methanol/glacial acetic acid (95:4:1, v/v/v) as mobile phase; in urine	[309]
Pyrimethamine, sulphadoxine	HPLC	In human plasma; after an automated liquid–solid extraction on a C_8 cartridge, the compounds are separated on a C_{18} column by isocratic elution; the mobile phase is methanol–acetonitrile–water (10:25:65, v/v/v) with triethylamine (1%) and adjusted to pH 5.6 with phosphoric acid. The eluent is monitored with an ultraviolet detector at 240 nm. Sulphadimethoxine is used as an internal standard	[310]
Sulphapyridine	HPLC	Indirectly indicative of gastric emptying and orocecal transit, respectively. Extraction of the drugs from serum was achieved with chloroform:isopropyl alcohol (7:3). Analysis was performed with a mobile phase comprising 1.9% tetrahydrofuran in 0.01 M sodium acetate buffer adjusted to pH 4.5 through a YMC-Packed C_{18} column at a flow rate of 1.0 ml min^{-1} and UV detection at 254 nm. The detection limit is 0.2 μg ml^{-1} for paracetamol and 0.1 μg ml^{-1} for sulphapyridine	[311]
–	Reversed-phase HPLC	In human serum; the time of proper analysis does not exceed 15 min. The linear response of the detector has been proved for the range of 1–80 mg l^{-1}	[312]
Acetanilide, phenacetin	GC–MS	By the use of deuterium labelled analogues; in plasma and urine	[313]
–	GC	Plasma proteins precipitated with sulphotungstic acid, supernatant liquid mixed with pyridine, PCT extracted by salting-out with sodium sulphate	[314]
Phenacetin	Capillary column GC–negative-ion MS	Phenacetin and unconjugated paracetamol are analysed in a single chromatographic run while total paracetamol is measured separately after enzymatic hydrolysis. The two compounds, and the deuterated analogues used as internal standards, are analysed as their trifluoroacetyl derivatives and the mass spectrometer is operated in the electron-capture negative-ion chemical ionisation mode	[315]

Other chromatographic methods proposed for determination of paracetamol in biological fluids are summarised in Table 6.

5. Capillary electrophoretic methods

Capillary electrophoresis (CE) is a very sensitive separation technique that has been developed based on the knowledge acquired from HPLC. CE allows the separation of biomolecules with high performance where HPLC fails. Certain CE methods are a hybrid between electrophoresis and chromatography, such as electrochromatography.

With respect to PCT, CE is used in diverse instances for resolving different questions. Therefore, in the last few years several CE methods have been developed for determination of PCT [316–318] using micellar electrokinetic capillary chromatography (MECC), which is a modification of CE combining the advantages of CE and chromatography. This technique is employed when PCT has to be separated effectively from plasma proteins [317]. However, these methods have not been used for separation of paracetamol glucuronide and paracetamol. For separation of glucuronides and their corresponding aglycones like morphine and its 3- and 6-glucuronide or 7-hydroxycoumarin and its glucuronide, there are some CE methods described in the literature, whereas no data are available dealing with the separation of paracetamol glucuronide and paracetamol. A CE method was developed using paracetamol glucuronide as a novel probe for human β-glucuronidase activity [319]. Using UV detection without prior sample clean-up procedures, fast and reliable quantitation of the released paracetamol was possible. The new drug composite pseudoephedrine

hydrochloride tablets are used for the treatment of the symptoms of the common cold and influenza. The tablet contains pseudoephedrine (PSED) as a vasoconstrictor for reducing nasal congestion, dextromethorphan hydrochloride (DEMP) as a cough suppressant and paracetamol as an analgesia and antipyresis. About PSED, PCT and DEMP, some analytical methods have been reported. The official analytical methods are based on titration for PSED, spectrophotometry and thin-layer chromatography for PRT and liquid chromatography for DEMP. But these methods are only suitable for the general determination in single ingredient of compounds. Several methods have appeared in other literatures, including TLC, GLC, HPLC and spectrophotometry for monitoring the mixture which contain PSED, PCT and DEMP. However, TLC and spectrophotometry need complex procedures and LC requiring some solvents might result in pollution and both of them consume long time to complete the separation. Therefore, it is necessary to develop a new analytical procedure, which can save separation time and decrease the cost of the determination.

CE offers possible advantages over LC in terms of separation time, solvent pollution and analysis expense. It has been reported as a powerful tool for a wide range of analysis, including many applications to the determination of drugs, such as the main component determinations, drug-related impurities estimations, chiral separations, etc. The monitoring of PCT and its metabolites in human body by CE has been reported [319–321], but there were no papers for the simultaneous determination of PSED, PCT and DEMP by CE. A simple, rapid and specific CE method has been developed for the separation and determination of PSED, PCT and DEMP with UV detection [322]. The CE was performed in 20 mmol l^{-1} sodium phosphate buffer solution (pH 7.0). The three ingredients were completely separated within 5 min, and quantified with an UV detector at 200 nm.

Also, a simple, reliable screening method was developed that allows identification of the drug and/or its metabolites in urine after oral application of paracetamol, acetylsalicylic acid, antipyrine, ibuprofen, naproxen, ketoprofen and propyphenazone by their migration in CE and by their UV spectra recorded with a diode-array detector in a common CE–UV system with 50 mM borax pH 9.4 as separation buffer [323]. For the CE–electrospray (ESI)–MS coupling a volatile 50 mM ammonium acetate buffer at pH 9.8 was used. In order to analyse the metabolic pattern in more detail different methods were developed for each drug. The separation of the metabolites of acetylsalicylic acid could be improved by injection of the urine sample at the cathodic side of the capillary. In order to identify antipyrine as neutral compound as well as its neutral metabolites a micellar electrokinetic chromatography (MEKC) method was developed.

During the past decade, considerable interest has been focused on micro-total analysis system (μTAS) or so called "lab-on-a-chip", and particular attention has been paid to capillary electrophoresis microchips due to its advantages over conventional analysis methods, such as rapid separation speed, high separation efficiency, low reagent consumption, reduced production of waste and use of energy and its potential portability and disposability. As yet, the μTAS has been developed, refined and applied to a variety of chemical and biological problem. The microfluidic devices developed in the early years were mostly fabricated from silicon and glass using photolithography and etching technique. However, these fabrication processes were costly, time-consuming, labour-intensive and clean-room conditions were required. The fabricated microfluidic devices are also fragile and mass production is not easy to be achieved. Recently, polymeric microchips are of increasing interest because they can offer attractive mechanical and chemical properties, low cost, ease of fabrication, biocompatibility and higher flexibility. Polymeric materials, including poly(dimethylsiloxane) (PDMS), poly(methyl methacrylate) (PMMA), polycarbonate, polystyrene (PS) and PET, have commonly been employed in the fabrication of microfluidic devices, such polymeric chips have been fabricated using laser ablation, plasma etching, imprinting, hot embossing, injection molding and compression molding techniques. Recently, it is described a very simple microfabrication process based on direct printing for mass production of microfluidic devices at very low cost. Compared to photolithography approaches, this process is an attractive alternative to other expensive, laborious and time-consuming methods for microchannels fabrication. However, it is found that such chips are not so durable during the operation procedures due to possible toner falling off the transparency in experiments. Therefore, it is proposed an improved method for fabricating PET microchips with high stability and durability. In this method, PET films with adhesive film were finally plastified on the microchips.

Since Mathies et al. developed a microchip-based CE–EC system for indirect electrochemical EC detection of DNA, EC detection has been offered great promise for designing self-contained and totally disposable μTAS. There are several advantages associated with EC detection such as extremely low cost, low power requirement, high selectivity, remarkable sensitivity, inherent miniaturisation and high compatibility advanced micromachining and microfabrication technologies. EC detection has been proven to be a promising detection method and the most widely reported for microchip. Rapid separation and determination of PCT and its hydrolysate with end-channel electrochemical (EC) detection integrated on a plastified poly(ethylene terephthalate) (PET)–toner microchip capillary electrophoresis system was proposed [324]. In this separation and detection system, a Pt ultramicroelectrode integrated on a three-dimensional adjustor was used as working electrode.

CE with UV detection has been applied for determination of paracetamol in tablets [325,326], and a CE/MS procedure has also been reported for determination of paracetamol and its metabolites in biological fluids [321]. Major drawbacks of CE with UV detection are its sensitivity. Chemiluminescence detection has been proven to be one of the most sensitive detection technologies, and the costs of the instrumental set-up for CL detection are relatively low. Therefore, CL detection has become an attractive detection scheme for sensitive detection in CE. Indirect detection of paracetamol was accomplished using a capillary electrophoresis–chemiluminescence (CE–CL) detection system, which was based on its inhibitory effect on a luminol–potassium hexacyanoferrate(III) ($K_3[Fe(CN)_6]$) CL

reaction [327]. PCT migrated in the separation capillary, where it mixed with luminol included in the running buffer. The separation capillary outlet was inserted into the reaction capillary to reach the detection window. A four-way plexiglass joint held the separation capillary and the reaction capillary in place. $K_3[Fe(CN)_6]$ solution was siphoned into a tee and flowed down to the detection window. CL was observed at the tip of the separation capillary outlet. The CL reaction of $K_3[Fe(CN)_6]$ oxidised luminol was employed to provide the high and constant background. Since PCT inhibits the CL reaction, an inverted PCT peak can be detected, and the degree of CL suppression is proportional to the PCT concentration. Maximum CL signal was observed with an electrophoretic buffer of 30 mM sodium borate (pH 9.4) containing 0.5 mM luminol and an oxidiser solution of 0.8 mM $K_3[Fe(CN)_6]$ in 100 mM NaOH solution.

Among the CE techniques employed for the analysis of ASA, PCT and CAF, interesting results were obtained using capillary electrochromatography (CEC) coupled with nuclear magnetic resonance (NMR) [328], however, the analytical method is very expensive and requires experience in the field.

The usefulness of CEC for the analysis of ASA or PCT was demonstrated by Altria et al. [329] and CAF in combination with other correlated substances and PCT using a normal-phase CEC [330]. CEC is a recently developed separation technique combining the best properties of capillary electrophoresis (high efficiency) with those of HPLC, i.e. high selectivity and increased sample loading which has recently generated great interest especially in the analytical field. In CEC the mobile phase and the analytes, under the influence of a relatively strong electric field, are driven to the detector through a capillary usually packed with a stationary phase employed in HPLC. Solutes are separated according to their partitioning coefficient between the two phases and, when charged, their electrophoretic mobility should also be considered. A strong electroosmotic flow (EOF) is necessary in order to speed up the CEC analysis and this can be easily obtained by using classical reversed-phase stationary phases where free silanol groups are present. As in CE, the EOF has a nearly flat flow velocity profile with reduced dispersion compared to LC, leading to a high efficiency. Reversed-phase CEC has been successfully used for the separation of neutral drugs and acidic compounds. The major factor holding back its further development has been the perceived inability of CEC to analyse basic compounds. In fact, the analysis of basic compounds often results in broad peaks and serious peak tailing. The same residual silanol groups that are essential for creating the EOF cause this tailing. Pharmaceuticals (and their impurities) are often basic, and, therefore, the application of CEC to pharmaceutical analysis can be often problematic. However, the addition of a competing amine to the mobile phase at low pH, has been proposed to improve the separation of basic substances by reversed-phase CEC.

The separation and simultaneous determination of caffeine, paracetamol and acetylsalicylic acid in two analgesic tablet formulations was investigated by capillary electrochromatography [331]. The effect of mobile phase composition on the separation and peak efficiency of the three analytes was studied and evaluated; in particular, the influence of buffer type, buffer pH and acetonitrile content of the mobile phase was investigated. The analyses were carried out under optimised separation conditions, using a full-packed silica capillary (75 μm i.d.; 30.0 and 21.5 cm total and effective lengths, respectively) with a 5 μm C_8 stationary phase. A mixture of 25 mM ammonium formate at pH 3.0 and acetonitrile (30:70, v/v) was used as the mobile phase. UV detection was at 210 nm.

6. Water analysis

Back in 1977, during water control studies, the penetration of the aquatic environment by pharmaceutical products and their metabolites became apparent. The pharmaceutical industry can be exonerated because of the stringent guidelines and waste control systems to which drug manufacture is subject. Nevertheless, the growing production of drugs for human healthcare and veterinary applications results in increasing amounts of highly biological active material being discharged into the environment. Nowadays it is known that the 'domestic' pathway, via municipal sewage treatment plants, is the main route of pharmaceutical substances into the aquatic environment. Due to their polarity, persistence and water solubility, most drugs and metabolites are able to pass through the wastewater treatment plants. Low adsorption on sludge and soil may cause the contamination of surface and ground water. Consequently, the quality of drinking water produced from this ground water or river bank filtrate may be endangered. The monitoring of wastewater effluents, surface and tap water has demonstrated the widespread distribution of pharmaceutical substances. Although detailed knowledge about the ecotoxicological effects of these compounds is still lacking, these contaminants must be classified as environmentally relevant.

The widespread occurrence of endocrine disrupting compounds and the consequences for the ecosystem are often discussed separately from pharmaceutical substances. Extensive ecotoxicological studies have revealed the variety of effects of hormone-disrupting chemicals on organisms. A wide range of industrially produced and applied chemicals, such as pesticides, alkylphenols formed from surfactants and plasticisers such as phthalates, display estrogenic effects. One of the main problems when collecting data about the occurrence, distribution and behaviour of biologically active substances in the environment is that trace analysis is required for a great variety of compounds. The polar, water-soluble pharmaceuticals are present in trace amounts, and in the case of surface water or wastewater, they are accompanied by complex organic matter. Nowadays, modern analytical methods combine sample enrichment (solid-phase extraction, SPE), derivatisation and GC–MS analysis using selected-ion monitoring for trace-level detection.

Investigations were often limited to the analysis of selected individual compounds, because the diverse range of analytes required highly specialised sample preparation and enrichment procedures. A solid-phase microextraction (SPME) method for determining trace amounts of polar, biologically active substances in water systems was developed and compared with solid-phase extraction followed by derivatisation and GC–MS [332]. SPME was examined with respect to the simultaneous

determination of pharmaceuticals such as ibuprofen, paracetamol, phenazone, carbamazepine and nonylphenols known to be xenoestrogens. The extraction performance of different SPME fibre coatings was studied. Coatings like polyacrylate and Carbowax–divinylbenzene proved to be the best suited. Methods for the determination of drug residues in water have been developed based on the combination of liquid chromatography (LC) or capillary electrophoresis with mass spectrometry (MS). For HPLC–MS two types of interfaces (pneumatically assisted electrospray ionisation interface or an atmospheric pressure chemical ionisation interface, respectively) were employed and compared in terms of detection limits [333]. Ammonium acetate (2 mM) at pH 5.5 and a methanol gradient was used for the HPLC–MS allowing the separation of a number of drugs such as paracetamol, clofibric acid, penicillin V, naproxen, bezafibrate, carbamazepine, diclofenac, ibuprofen and mefenamic acid. A 20 mM ammonium acetate solution, pH 5.1 was employed for the separation of clofibric acid, naproxen, bezafibrate, diclofenac, ibuprofen and mefenamic acid by CE–MS. Sample pre-treatment was performed by solid-phase extraction for HPLC–MS or by a combination of liquid–liquid extraction and SPE for CE–MS. The applicability of both the HPLC–MS and CE–MS method was demonstrated for several river water samples.

A simple method is presented for the analysis of 13 pharmaceutical and pharmaceutical metabolite compounds in sewage effluents and surface waters [334]. The pharmaceutical compounds were extracted using a generic solid-phase extraction procedure using Phenomenex Strata X as a stationary phase. Extracts were quantitatively analysed by four separate reversed-phase high performance liquid chromatography–electrospray tandem mass spectrometry (HPLC–ESI–MS/MS) techniques and quantified by comparison with an internal standard ([^{13}C]-phenacetin).

7. Conclusions

The first and principal conclusion is that there is very much papers that report the determination of paracetamol in formulations in comparison to the papers where it is described the determination of this compound in biological fluids.

Numerous methods have been used for the determination of paracetamol in pharmaceutical formulations including titrimetry, UV/vis spectrophotometry, spectrofluorimetry, near infrared transmittance spectroscopy and others. Recently, several flow-injection (FI) methods for the determination of paracetamol have been proposed using an UV/vis spectrophotometer, fluorimeter, multioptosensor or Fourier transform infrared spectrophotometer as a detector. However, some of these methods are less convenient for the determination of paracetamol in pharmaceutical formulations because the methods are based on the hydrolysis of paracetamol sample to 4-aminophenol, which then produced a coloured complex compound by an appropriate reaction which may increase the reagent consumption, time required for analysis and possibility of sample contamination. A sample preparation step prior to the determination makes the above-mentioned procedures somewhat laborious and less attractive for multisample analyses. This is often a limiting factor in the chemical and pharmaceutical industries, where a large number of samples must be assayed for quality control purposes. Simpler procedures performed in a shorter time are preferred and analytical procedures that do not involve use of chemicals and/or sample pre-treatment steps are then a good alternative. In this context, a proposed alternative is FI with CL.

Also, near infrared and FT-Raman spectroscopy have been used for paracetamol determination in the solid phase. However, quantitative determinations with these techniques are only feasible by applying chemometric algorithms, especially in relation to NIR, where the spectra do not have high resolution, and complex-calibrating models must be constructed. In this way, spectrofluorimetry in the UV/vis region can be used to perform the measurements directly in the solid matrix, leading to favourable characteristics.

On the other hand, the resolution of the mixtures containing two or more different analytes without using a chemical separation and a graphical procedure is one of the main problems of the classical analytical chemistry. With the development of chemometric techniques such as classical least-squares, inverse least-squares, partial least-squares and principal least component regression, a lot of the problems of the simultaneous analysis of two or multicomponent mixtures have been solved. Although these methods are very easy to apply to spectrophotometric, chromatographic and electrochemical quantitative analysis, they require data processing with powerful softwares as well as the manipulation of the abstract vector space and its application to regression analysis.

Also, in the spectrophotometric studies, normal derivative and ratio spectra derivative spectrophotometry have been used for the quantitative resolving of the binary and ternary mixtures of paracetamol. Unfortunately, in some cases these two methods have a great disadvantage: higher derivative process diminishes the peak amplitude and it is difficult to find zero-crossing points. For this reason, the sensitivity of the method decreases. On the other hand, the ratio spectra derivative method leads us to an infinite value of ratio spectra in some cases.

With respect to the determination of PCT in biological fluids, there are few methods in the literature. The determination of paracetamol and its metabolites in biological fluids (urine, plasma and serum) has usually been carried out using either chromatographic or electrophoretic techniques. Conventional photometric and fluorimetric methods have occasionally been reported for the determination of paracetamol and its metabolites in biological fluids, as they are preferred for the determination of this compound in pharmaceuticals.

Urinary screening of paracetamol is usually carried out using either acid or enzymatic hydrolysis of the analyte to yield *p*-aminophenol; there is growing interest in developing rapid response analytical systems devoted to the determination of clinical parameters, as the result obtained will condition the course of treatment to be administered to the patient. A sample screening system provides a binary yes/no response, which indicates if the target analytes are present above or below a pre-set concentration; their potential in analytical chemistry has been recently pointed out. The most favourable situation

Table 7
Comparison of diverse methods for the determination for paracetamol

Method	Calibration range	Detection limit	Recovery (%)	R.S.D. (%)	Reference
HPLC	1.5–200 $\mu g\,ml^{-1}$	50 $ng\,ml^{-1}$	97.9–102.8	0.2–12.3	[292]
Flow-through UV optosensing device	0.5–8.0 $\mu g\,ml^{-1}$	0.022 $\mu g\,ml^{-1}$	–	1.24	[94]
HPCE	1–100 μM	0.5 μM	–	1.4–2.07	[317]
HPTLC	120–360 $\mu g\,ml^{-1}$	1.8 $\mu g\,ml^{-1}$	96–103	–	[255]
MBKC	260–520 $\mu g\,ml^{-1}$	0.5 $\mu g\,ml^{-1}$	95–103	0.47–0.9	[258]
Flow solid-phase UV spectrophotometry	3–30 $\mu g\,ml^{-1}$	0.104 $\mu g\,ml^{-1}$	Close to 100	0.6	[95]
HPLC and SFC	0.3–20 $\mu g\,ml^{-1}$	0.1–0.2 $\mu g\,ml^{-1}$	>99.2	2.69	[267]
IC	0.5–7 $mg\,l^{-1}$	0.06 $mg\,l^{-1}$	–	–	[244]
FTIR spectrometry	2–10 $mg\,l^{-1}$	0.34 $mg\,l^{-1}$	–	–	[158]
CE	0.5–100 μM	0.25 μM	–	2.4–11.8	[319]
MIP–BAW sensor	5.0×10^{-3} to 0.1 μM	5.0×10^{-3} μM	93.3–106.7	4.69–6.55	[212]

is when the screening method can be used with no sample pre-treatment.

Many analytical methods are employed for quality control, stability testing, identification and clinical studies before bringing a drug product from the discovery stage to the commercial market. Analysts choose from a number of techniques, such as NMR, MS, separations, spectroscopy and electrochemistry, each having its own advantages. Electrochemistry has many advantages making it an appealing choice for pharmaceutical analysis. Most electrochemical techniques have excellent detection limits and a wide dynamic range. The role of electrochemistry in pharmaceutical analysis has been well defined, and is likely to get preference when low analyte concentrations, small sample volumes or complex sample matrices requiring high specificity challenge the analytical method.

Comparison of the results of the determination of paracetamol obtained by several methods is shown in Table 7. Compared with several current techniques for the assay of paracetamol, the MIP–BAW sensor has some advantages. It has the same specific recognition selectivity as immunoassay but with the advantage of stability and cheap reagents. Furthermore, the instruments of the MIP–BAW sensor are simpler and cheaper than that of high powered liquid chromatography (HPLC), CE and IC. At the same time, the procedure and pre-treatment for the MIP sensor are simple and easy. Also, flow-injection analysis with biamperometric detection offers the advantages of the selectivity of biamperometry due to low applied potentials and those from the FIA methodology like increased reproducibility and sample throughput of the inherently lower dispersion of FIA.

On the other hand, HPLC methods are simple, rapid and sensitive and therefore suitable for the routine analysis of PCT and other compounds presents in multidrug pharmaceutical preparations. Most methods for determining paracetamol in biological samples use HPLC or gas chromatography. Other methods include enzymatic and colorimetric techniques. Methods that use a small volume of blood are desirable in situations where the collection of larger volumes is not feasible. Also, other features which are desirable are simplicity of sample preparation and general availability of the analytical instrument required for analysis.

Studies involving the disposition of paracetamol in humans frequently require a sensitive, accurate and precise assay that is capable of quantifying paracetamol and its major metabolites within the same analytical run in both plasma and urine. Very few analytical methods are available that measure the two major metabolites of paracetamol directly and even fewer have the accuracy, precision and sensitivity to analyse samples from single-dose pharmacokinetic studies. HPLC is also used for measurement of the concentrations of paracetamol and its two major metabolites simultaneously in plasma and in urine. An additional advantage of the method is that concentrations in low volumes of plasma obtained from a finger-prick may be measured with sufficient sensitivity to characterise the concentrations in plasma versus time profiles over almost two orders of magnitude.

Finally, the capabilities of CE for the determination of drugs and their metabolites in body fluids are demonstrated. In urine samples, paracetamol and its main compounds could be determined directly by a simple, rapid and reliable assay. In particular, the good separations of the conjugates reveal the advantages of this analytical method for the investigation of metabolites. The injection of a water zone behind urine samples prevented the peaks from tailing and, thus, supported the direct determination in complex media like urine. The use of CE–MS and CE–DAD on-line detection accelerates and ensures peak identification in electropherograms, especially when urine samples of patients with co-medications, like patients under anticancer therapy, have to be analysed. In order to analyse paracetamol and its metabolites in serum, ultrafiltration was found to be the most suitable preparation to solve the problems that arose.

References

[1] Z.E. Kalinowska, H. Hasztar, Farm. Pol. 23 (1967) 447–450.
[2] British Pharmacopoeia CD, Version 2, The Stationery Office Ltd., Norwich, 1998.
[3] M.I. Walash, M. Rizk, Indian J. Pharm. 39 (1977) 82–83.
[4] S.A. Mohmoud, S.F. Abdel, N. Abdine, J. Assoc. Off. Anal. Chem. 34 (1979) 569–572.
[5] H. Affalion, N. Kein, M. Strerescue, Rev. Chim. 11 (1982) 14–18.
[6] K. Satiadarma, S. Tino, T. Hermini, Acta Pharm. Indones. 10 (1985) 37–38.
[7] M.K. Srivastava, D. Singh, S. Ahamed, I.S. Shukla, Zh. Prikl. Khim. 57 (1984) 901–904.
[8] V. Koen, Farmatsiya 15 (1965) 105–110.
[9] European Pharmacopoeia, 1997, Convention on the Elaboration of a European Pharmacopoeia, third ed., European Treaty Series No. 50, Strasbourg, 1996, pp. 748–749.

[10] B.C. Verma, D.K. Sharma, B.H.G. Rao, V.S. Jamwal, Indian J. Pharm. Sci. 54 (1992) 62–65.
[11] J. Hunt, H.J. Rhodes, M.I. Blake, Can. J. Pharm. Sci. 6 (1971) 20–23.
[12] M.I. Walash, S.P. Agarwal, M.I. Martin, Can. J. Pharm. Sci. 7 (1972) 123–127.
[13] S.P. Agarwal, M.I. Walash, Indian J. Pharm. 36 (1974) 47–49.
[14] M.C. Inamdar, J.C. Saboo, C.N. Kamdar, N.M. Sanghavi, Indian J. Pharm. 35 (1973) 187–189.
[15] C. Burgot, F. Auffret, J.L. Burgot, Anal. Chim. Acta 343 (1997) 125–128.
[16] M.K. Srivastava, S. Ahmad, D. Singh, I.C. Shukla, Analyst 110 (1985) 735–737.
[17] P. Parimoo, R.R. Sethuraman, A. Amalraj, N. Seshadri, Indian Drugs 26 (1989) 704–705.
[18] P. Umapathi, Indian Drugs 30 (1993) 596–599.
[19] K.G. Kumar, R. Letha, J. Pharm. Biomed. Anal. 15 (1997) 1725–1728.
[20] J.W. Murfin, Analyst 97 (1972) 663–669.
[21] J.W. Murfin, J.S. Wragg, Analyst 97 (1972) 670–675.
[22] D.R. Davis, A.G. Fogg, D. Thorburn, J.S. Wragg, Analyst 99 (1974) 12–18.
[23] J.W. Murfin, H. Dedicoat, J. Assoc. Pub. Anal. 11 (1973) 108–111.
[24] H. Dedicoat, D.C. Symonds, J. Assoc. Pub. Anal. 10 (1972) 14–17.
[25] C.O. Usifoh, S.A. Adelusi, R.F. Adebambo, Pakistan J. Sci. Ind. Res. 45 (2002) 7–9.
[26] B.K. Datta, S.C. Bachar, R. Banoo, S. Haque, Indian J. Pharm. Sci. 51 (1989) 270–271.
[27] H.L. Ederma, J. Skerpac, V.F. Cotty, F. Sterbenz, Technicon Symposium 1966, vol. I, Mediad Inc., New York, 1967, p. 228.
[28] D. Horn, Pharm. Zentralhalle Dtl. 90 (1951) 296–297.
[29] B.N. Afanas'ev, Aptech. Delo. 2 (1953) 21–23.
[30] C.T.H. Ellcock, A.G. Fogg, Analyst 100 (1975) 16–18.
[31] A.A. El Kheir, S. Belal, M. El Sadek, A. El Shanwani, Analyst 111 (1986) 319–321.
[32] M. El Sadek, Anal. Lett. 19 (1986) 479–488.
[33] British Pharmacopoeia, The Stationery Office, London, 1999.
[34] United States Pharmacopeia, 24th revision, United States Pharmacopeial Convention, Rockville, 2000.
[35] J. Ruzicka, E. Hansen, Flow Injection Analysis, second ed., Wiley, New York, 1988.
[36] M. Trojanowicz, Flow Injection Analysis: Instrumentation and Applications, World Scientific, Singapore, 2000.
[37] J. Martínez-Calatayud, Flow Injection Analysis of Pharmaceuticals-Automation in the Laboratory, Taylor and Francis, London, 1996.
[38] J. Martínez-Calatayud, M.C. Pascual-Martí, S. Sagrado-Vives, Anal. Lett. 19 (1986) 2023–2038.
[39] Z. Bouhsain, S. Garrigues, A. Morales-Rubio, M. De la Guardia, Anal. Chim. Acta 330 (1996) 59–69.
[40] A. Criado, S. Cárdenas, M. Gallego, M. Valcárcel, Talanta 53 (2000) 417–423.
[41] F. Le Perdriel, C. Hanegraff, N. Chastagner, E. De Montety, Ann. Pharm. Fr. 26 (1968) 227–237.
[42] L. Chafetz, R.E. Daly, H. Schriftman, J. Lomner, J. Pharm. Sci. 60 (1971) 463–466.
[43] M. Knochen, J. Giglio, B.F. Reis, J. Pharm. Biomed. Anal. 33 (2003) 191–197.
[44] A.A. D'Souza, K.G. Shenoy, Can. J. Pharm. Sci. 36 (1974) 47–49.
[45] N.M. Sanghavi, D.R. Vishwasrao, Indian J. Pharm. 35 (1973) 172–173.
[46] T. Inoue, M. Tatsuzawa, S. Lee, T. Ishii, Eisei Kagaku 21 (1975) 313–317.
[47] F.M. Plakoviannis, A.M. Saad, J. Pharm. Sci. 64 (1975) 1547–1549.
[48] E. Kalatzis, I. Zarbi, J. Pharm. Sci. 65 (1976) 71–75.
[49] M.C. Inamdar, M.S. Gore, R.V. Bhide, Indian J. Pharm. 36 (1974) 7–9.
[50] L.C. Goncalves, Bol. Es. Fac. Farm. Univ. Coimbra, Ed. Didact. Not. Farm. 40 (1974) 2–4.
[51] S.F. Belal, M.A.H. Elsayed, A. Elwalily, H. Abdine, Analyst 104 (1979) 919–927.
[52] British Pharmacopoeia, H.M. Stationery Office, London, 1973, p. 340.
[53] M.A.H. Elsayed, S.F. Belal, A. Elwalily, H. Abdine, Analyst 104 (1979) 620–625.
[54] K.K. Verma, A.K. Gulati, S. Palod, P. Tyagi, Analyst 109 (1984) 735–737.
[55] S.A. Al-Tamrah, Oriental J. Chem. 3 (1987) 145–151.
[56] S.Z. Qureshi, A. Saeed, Ann. Chim. 80 (1990) 203–207.
[57] K. Lavanya, T.R. Baggi, Indian Drugs 26 (1989) 440–441.
[58] S.M. Sultan, I.Z. Alzamil, A.M. Aziz Alrahman, S.A. Altamrah, Y. Asha, Analyst 111 (1986) 919–921.
[59] L. Davey, D. Naidoo, Clin. Chem. 39 (1993) 2348–2349.
[60] C.S.P. Sastry, K.V.S.S. Murthy, Indian Drugs 19 (1982) 158–161.
[61] K.K. Verma, A. Jain, Talanta 32 (1985) 238–240.
[62] S.M. Sultan, Talanta 34 (1987) 605–608.
[63] K.P.R. Chowdary, G.D. Rao, Eastern Pharma. 40 (1997) 151–152.
[64] S.Z. Qureshi, A. Saeed, N. Rahman, Chem. Anal. 37 (1992) 227–229.
[65] E.S.M. Nameh, M.I.H. Helaleh, T. Korenaga, M.A.Q. Jamhoor, N. Suharto, Acta Poloniae Pharm. 56 (1999) 407–411.
[66] M.I.H. Helaleh, T. Korenaga, E.S.M. Abu-Nameh, R.M.A.Q. Jamhour, Pharm. Acta Helv. 73 (1999) 255–260.
[67] P. Nagaraja, K.C. Srinivasa Murthy, K.S. Rangappa, J. Pharm. Biomed. Anal. 17 (1998) 501–506.
[68] F.A. Mohamed, M.A. AbdAllah, S.M. Shammat, Talanta 44 (1997) 61–68.
[69] A.S. Amin, Sci. Pharm. 69 (2001) 179–188.
[70] P.K. Malik, S.K. Sanyal, S.K. Bhowal, J. Surf. Sci. Technol. 17 (2001) 75–83.
[71] G. López-Cueto, M. Ostra, C. Ubide, Anal. Chim. Acta 445 (2001) 117–126.
[72] C. Xu, B. Li, Spectrochim. Acta Part A 60 (2004) 1861–1864.
[73] B. Morelli, J. Pharm. Biomed. Anal. 7 (1989) 577–584.
[74] P.B. Issopoutos, Anal. Lett. 23 (1990) 1057–1061.
[75] M.A. Korani, D. Heber, J. Schnekenburger, Talanta 29 (1982) 332–334.
[76] M. Anwar Memon, M. Aslam Memon, M. Umar Dahot, Sci. Int. 7 (1995) 55–59.
[77] M. Abdel-Hady Elsayed, S.F. Belal, M.E. Abdel-Fattah, H. Abdine, Analyst 104 (1979) 620–625.
[78] A.S. Issa, Y.A. Beltagy, M. Gabr Kassem, H.G. Daabees, Talanta 32 (1985) 209–211.
[79] S.D. Prasad, Indian Drugs 19 (1991) 130–131.
[80] A.S. Amin, Quim. Anal. 19 (2000) 135–138.
[81] A.S. Amin, M.Y. El-Maamly, Quim. Anal. 20 (2002) 275–279.
[82] A. Safavi, O. Moradlou, Anal. Lett. 37 (2004) 2337–2349.
[83] M. Carmona, M. Silva, D. Perez-Bendito, Fresenius Z. Anal. Chem. 334 (1989) 261–265.
[84] D.J. Saheb, N.R. Reddy, I.E. Chakravarthy, Asian J. Chem. 16 (2004) 767–772.
[85] D. Mundhe, S.G. Kaskhedikar, Eastern Pharma. 38 (1995) 181–182.
[86] A.M.I. Mohamed, P.Y. Khashaba, H.A. Mohamed, Saudi Pharm. J. 2 (1994) 42–46.
[87] H. Filik, M. Hayvali, E. Kilic, Anal. Chim. Acta 535 (2005) 177–182.
[88] S.D. Cekic, H. Filik, R. Apak, J. Anal. Chem. 60 (2005) 1019–1023.
[89] K.K. Verma, A. Jain, K.K. Stewart, Anal. Chim. Acta 261 (1992) 261–267.
[90] C.A. Georgiou, M.A. Koupparis, Analyst 115 (1990) 309–313.
[91] J. Martinez-Calatayud, S. Sagrado-Vives, J. Pharm. Biomed. Anal. 7 (1989) 1165–1172.
[92] A. Criado, S. Cárdenas, M. Gallego, M. Valcárcel, Analyst 125 (2000) 1179–1183.
[93] S. Dunkerley, M.J. Adams, Lab. Autom. Inf. Manag. 33 (1997) 107–117.
[94] M.J. Ayora-Cañada, M.I. Pascual-Reguera, A. Ruiz Medina, M.L. Fernández de Córdova, A. Molina Díaz, J. Pharm. Biomed. Anal. 22 (2000) 59–66.
[95] A. Ruiz Medina, M.L. Fernández de Córdova, A. Molina Díaz, Anal. Chim. Acta 394 (1999) 149–158.
[96] A. Ruiz Medina, M.L. Fernández de Córdova, M.J. Ayora-Cañada, M.I. Pascual-Reguera, A. Molina Díaz, Anal. Chim. Acta 404 (2000) 131–139.
[97] J.M. Calatayud, M.C. Pascual-Marti, S. Sagrado-Vives, Anal. Lett. 19 (1986) 2023–2038.
[98] C. Aniceto, O. Fatibello-Filho, Quim. Nova 25 (2002) 387–391.

[99] R. Burakham, S. Duangthong, L. Patimapornlert, N. Lenghor, S. Kasiwad, L. Srivichal, S. Lapanantnoppakhun, J. Jakmunee, K. Grudpan, Anal. Sci. 20 (2004) 837–840.
[100] J. Martinez-Calatayud, S. Sagrado Vives, C. Gomez Benito, Quim. Anal. 8 (1989) 455–461.
[101] P. Ortega-Barrales, R. Padilla-Weigand, A. Molina-Diaz, Anal. Sci. 18 (2002) 1241–1246.
[102] A.D. Vidal, J.F.G. Reyes, P.O. Barrales, A.M. Diaz, Anal. Lett. 35 (2002) 2433–2447.
[103] A.D. Vidal, P.O. Barrales, A.M. Diaz, Microchim. Acta 141 (2003) 157–163.
[104] J.F. van Staden, M. Tsanwani, Talanta 58 (2002) 1095–1101.
[105] R. Burakham, S. Duangthong, L. Patimapornlert, N. Lenghor, S. Kasiwad, L. Srivichai, S. Lapanantnoppakhun, J. Jakmunee, K. Grudpan, Anal. Sci. 20 (2004) 837–840.
[106] W.T. Suarez, H.J. Vieira, O. Fatibello-Filho, Ecletica Quim. 30 (2005) 21–28.
[107] F. Priego-Capote, M.D. Luque de Castro, Anal. Chim. Acta 489 (2003) 223–232.
[108] A. Oezdemir, E. Dinc, F. Onur, Turkish J. Pharm. Sci. 1 (2004) 139–151.
[109] I.M. Palabiyik, U. Ustundag, E. Dinc, F. Onur, Turkish J. Pharm. Sci. 1 (2004) 1–15.
[110] A. Van Loosbroek, H.J.G. Debets, P.M.J. Coenegracht, Anal. Lett. 17 (1984) 779–792.
[111] G. Sala, S. Maspoch, H. Iturriaga, M. Blanco, V. Cerda, J. Pharm. Biomed. Anal. 6 (1988) 765–772.
[112] L.J. Rogers, M.J. Adams, Anal. Commun. 33 (1996) 401–402.
[113] H. Shen, S. Cai, Gaodeng Xuexiao Huaxue Xuebao 14 (1993) 918–921.
[114] H. Shen, S. Cai, Fenxi Huaxue 22 (1994) 716–718.
[115] Z. Bouhsain, S. Garrigues, M. de la Guardia, Fresenius J. Anal. Chem. 357 (1997) 973–976.
[116] C. Arama, C. Georgita, Farmacia 50 (2002) 30–33.
[117] D. Basu, K.K. Mahalanabis, B. Roy, J. Pharm. Biomed. Anal. 16 (1998) 809–812.
[118] G. Ragno, G. Iole, A. Risoli, Anal. Chim. Acta 512 (2004) 173–180.
[119] A. Ruiz Medina, M.L. Fernandez de Córdova, A. Molina Diaz, J. Pharm. Biomed. Anal. 21 (1999) 983–992.
[120] H.C. Goicoechea, A.C. Olivieri, J. Pharm. Biomed. Anal. 20 (1999) 255–261.
[121] A. Bozdogan, G.K. Kunt, A.M. Acar, Anal. Lett. 25 (1992) 2051–2058.
[122] A. Bozdogan, A.M. Acar, G.K. Kunt, H. Caglar, Pharmazie 49 (1994) 457–458.
[123] Y. Ni, C. Liu, S. Kokot, Anal. Chim. Acta 419 (2000) 185–196.
[124] E. Dinc, J. Pharm. Biomed. Anal. 33 (2003) 605–615.
[125] E. Dinc, A. Ozdemir, Pharmazie 59 (2004) 700–705.
[126] M.M. Sena, R.J. Poppi, J. Pharm. Biomed. Anal. 34 (2004) 27–34.
[127] A. Afkhami, M. Bahram, Talanta 66 (2005) 712–720.
[128] M.C. Pascual-Marti, A. Cerdan-Vidal, M. Llobat-Estelles, Microchem. J. 49 (1994) 36–47.
[129] M. Ustun, S. Sungur, L. Ersoy, Pharmazie 47 (1992) 558–559.
[130] N. Erk, F. Onur, Anal. Lett. 30 (1997) 1201–1210.
[131] A. Dimitrovska, S. Trajkovic-Jolevska, K. Stojanoski, Anal. Lab. 7 (1998) 32–35.
[132] G. Yurdakul, L. Ersoy, S. Sungur, Pharmazie 46 (1991) 885.
[133] E. Dinc, J. Pharm. Biomed. Anal. 21 (1999) 723–730.
[134] M.I. Toral, P. Richtery, O. Martinez, Bol. Soc. Chilena Quim. 41 (1996) 283–289.
[135] M.U. Ozgur, S. Sungur, Turkish J. Med. Sci. 25 (1995) 101–104.
[136] O.M. Uestuen, S. Sungur, Chim. Acta Turc. 23 (1995) 119–125.
[137] N. Erk, Sci. Pharm. 64 (1996) 173–183.
[138] E. Dinc, C. Yücesoy, F. Onur, J. Pharm. Biomed. Anal. 28 (2002) 1091–1100.
[139] M. Acikkol, Marmara Univ. Eczacilik Dergisi 6 (1990) 91–97.
[140] N. Erk, Y. Özkan, E. Banoglu, S.A. Özkan, Z. Sentürk, J. Pharm. Biomed. Anal. 24 (2001) 469–475.
[141] S. Kir, C. Safak, A. Tureli, A. Temizer, Fresenius J. Anal. Chem. 339 (1991) 264.
[142] C. Yucesoy, K. Tulek, Egyptian J. Anal. Chem. 3 (1994) 156–159.
[143] F. Onur, N. Acar, Gazi Univ. Eczacilik Fakultesi Dergisi 6 (1989) 23–30.
[144] U.G. Gonullu, N. Erk, Anal. Lett. 32 (1999) 2625–2639.
[145] F. Onur, N. Acar, Analysis 18 (1990) 560–561.
[146] C. Coiffard, L. Coiffard, Y. De Roeck-Holtzhauer, Ann. Pharm. Francaises 56 (1998) 229–232.
[147] V. Rodenas, M.S. García, C. Sánchez-Pedreño, M.I. Albero, Talanta 52 (2000) 517–523.
[148] E. Dinc, F. Onur, Anal. Chim. Acta 359 (1998) 93–106.
[149] E. Dinc, Talanta 48 (1999) 1145–1157.
[150] E. Dinc, G. Kökdil, F. Onur, J. Pharm. Biomed. Anal. 26 (2001) 769–778.
[151] E. Dinc, A. Özdemir, D. Baleanu, Talanta 65 (2005) 36–47.
[152] A. Eustaquio, P. Gram, R.D. Jee, A.C. Moffatt, A.D. Trafford, Analyst 123 (1998) 2303–2306.
[153] A. Eustaquio, M. Blanco, R.D. Jee, A.C. Moffatt, Anal. Chim. Acta 383 (1999) 283–290.
[154] Y. Dou, Y. Sun, Y. Ren, Y. Ren, Anal. Chim. Acta 528 (2005) 55–61.
[155] Y. Dou, Y. Sun, Y. Ren, P. Ju, Y. Ren, J. Pharm. Biomed. Anal. 37 (2005) 543–549.
[156] D.A. Zoltan, L. Milena, M. Sylvia, K. Imre, Acta Pharm. Hungarita 75 (2005) 141–145.
[157] M. Blanco, M. Alcala, Eur. J. Pharm. Sci. 27 (2006) 280–286.
[158] V. Tantishaiyakul, N. Phadoongsombut, S. Kamaung, S. Wongwisansri, P. Mathurod, Pharmazie 54 (1999) 111–114.
[159] Z. Bouhsain, S. Garrigues, M. de la Guardia, Analyst 121 (1996) 635–639.
[160] Z. Bouhsain, S. Garrigues, M. de la Guardia, Analyst 121 (1996) 1935–1938.
[161] M.L. Ramos, J.F. Tyson, D.J. Curran, Anal. Proc. 32 (1995) 175–177.
[162] M.L. Ramos, J.F. Tyson, D.J. Curran, Anal. Chim. Acta 364 (1998) 107–116.
[163] N. Al-Zoubi, J.E. Koundourellis, S. Malamataris, J. Pharm. Biomed. Anal. 29 (2002) 459–467.
[164] R. Szostak, S. Mazurek, Analyst 127 (2002) 144–148.
[165] T. Kaito, K. Sagara, T. Yoshida, Y. Ito, Yakugaku Zasshi 94 (1974) 633–638.
[166] G. Amann, G. Gübitz, R.W. Frei, W. Santi, Anal. Chim. Acta 116 (1980) 119–125.
[167] H. Nakamura, Z. Tamura, Anal. Chem. 52 (1980) 2087–2092.
[168] A. Öztunc, Sci. Pharm. 54 (1986) 111–113.
[169] J. Shibasaki, R. Konishi, K. Yamada, S. Matsuda, Chem. Phar. Bull. 30 (1982) 358–361.
[170] J. Shibasaki, R. Konishi, K. Yamada, Chem. Phar. Bull. 28 (1980) 669–672.
[171] J. Martinez, C. Gomez, Anal. Chim. Acta 231 (1990) 259–264.
[172] J.A. Murillo Pulgarin, L.F. Garcia Bermejo, Anal. Chim. Acta 333 (1996) 59–69.
[173] J.A. Murillo Pulgarin, L.F. Garcia Bermejo, Anal. Lett. 29 (1996) 423–438.
[174] M.A. Oliva, R.A. Olsina, A.N. Masi, Talanta 66 (2005) 229–235.
[175] A.B. Moreira, H.P.M. Oliveira, T.D.Z. Atvars, I.L.T. Dias, G.O. Neto, E.A.G. Zagatto, L.T. Kubota, Anal. Chim. Acta 359 (2005) 257–261.
[176] I.I. Koukli, A.C. Calokerinos, T.P. Hadjiioannou, Analyst 114 (1989) 711–714.
[177] A. Gregorio Alapont, L. Lahuerta Zamora, J. Martínez Calatayud, J. Pharm. Biomed. Anal. 21 (1999) 311–317.
[178] D. Easwaramoorthy, Y.C. Yu, H.J. Huang, Anal. Chim. Acta 439 (2001) 95–100.
[179] W. Ruengsitagoon, S. Liawruangrath, A. Townshend, Talanta, in press.
[180] J.L. Vilchez, R. Blanc, R. Avidad, A. Navalón, J. Pharm. Biomed. Anal. 13 (1995) 1119–1125.
[181] J.E. Ray, J. Stove, K.M. Williams, Clin. Chem. 33 (1987) 718.
[182] A. Dasgupta, G. Kinnaman, Clin. Chem. 39 (1993) 2349–2350.
[183] P.M. Hammond, M.D. Scawen, T. Atkinson, R.S. Campbell, P. Price, Anal. Biochem. 143 (1984) 152–157.
[184] P.A.D. Edwardson, J.D. Nichols, K. Sugden, J. Pharm. Biomed. Anal. 7 (1989) 287–293.
[185] A. Criado, S. Cárdenas, M. Gallego, M. Valcárcel, Analyst 125 (2000) 1179–1183.

[186] A. Criado, S. Cárdenas, M. Gallego, M. Valcárcel, Anal. Chim. Acta 435 (2001) 281–288.
[187] J.T. Afshari, T. Liu, Anal. Chim. Acta 443 (2001) 165–169.
[188] R.T. Sane, S.S. Kamat, Curr. Sci. 49 (1980) 650–652.
[189] H.V. Patel, D.J. Morton, J. Clin. Pharm. Ther. 13 (1988) 233–238.
[190] M. Dolegeal-Vendrely, M. Guernet, Analysis 4 (1976) 223–226.
[191] P. Freycon, M. Accominotti, J.J. Vallon, Anal. Lett. 14 (1981) 1767–1782.
[192] A.M. Itinose, R.B. Sznelwar, Rev. Farm. Bioquim. Univ. Sao Paulo 18 (1982) 164–176.
[193] A.R. Zarei, A. Afkhami, N. Sarlak, J. AOAC Int. 88 (2005) 1695–1701.
[194] P.C. Damiani, M.E. Ribone, A.C. Olivieri, Anal. Lett. 28 (1995) 2219–2226.
[195] A. Concheiro, J.L. Vila, M. Llabres, Ciencia Ind. Farm. 1 (1982) 38–41.
[196] M. El Mouelhi, B. Buszewski, J. Pharm. Biomed. Anal. 8 (1990) 651–653.
[197] J. Parojcic, K. Karljikovic-Rajic, Z. Duric, M. Jovanovic, S. Ibric, Biopharm. Drugs Dispos. 24 (2003) 309–314.
[198] A. Di Girolamo, W.M. O'Neill, I.W. Wainer, J. Pharm. Biomed. Anal. 17 (1998) 1191–1197.
[199] J.W. Munson, R. Weierstall, H.B. Kostenbauer, J. Chromatogr. 145 (1978) 328–331.
[200] H. Bramwell, A.E. Cass, P.N. Giggs, M.J. Green, Analyst 115 (1990) 185–188.
[201] P.A. Vaughan, L.D.L. Scott, J.F. McAller, Anal. Chim. Acta 248 (1991) 361–365.
[202] J. Wang, T. Golden, P. Tuzhi, Anal. Chem. 59 (1987) 740–744.
[203] M.A.T. Gilmartin, J.P. Hart, Analyst 119 (1994) 2431–2437.
[204] A. Falkowski, R. Wei, Anal. Lett. 14 (1981) 1003–1012.
[205] J. Wang, T. Golden, Anal. Chim. Acta 217 (1989) 343–351.
[206] J. Wang, D. Hutchins, Anal. Chem. 57 (1985) 1536–1541.
[207] J.C. Apostolakis, C.A. Georgiou, M.A. Koupparis, Analyst 16 (1991) 233–237.
[208] J. Wang, H.D. Dewald, Anal. Chim. Acta 153 (1983) 325–330.
[209] A. Moreno Gálvez, J.V. García Mateo, J. Martínez Calatayud, Anal. Chim. Acta 396 (1999) 161–170.
[210] G.A. Messina, I.E. De Vito, J. Raba, Anal. Chim. Acta 559 (2006) 152–158.
[211] O. Fatibello-Filho, K. Omuro Lupetti, I. Cruz Vieira, Talanta 55 (2001) 685–692.
[212] Y. Tan, Z. Zhou, P. Wang, L. Nie, S. Yao, Talanta 55 (2001) 337–347.
[213] S. Bi, G. Wang, Y. Piao, D. Wang, X. Yin, Yanbian Daxue Xuebao, Ziran Kexueban 26 (2000) 110–114.
[214] J.M. Zen, Y.S. Ting, Anal. Chim. Acta 342 (1997) 175–180.
[215] F.Y. He, A.L. Liu, X.H. Xia, Anal. Bioanal. Chem. 379 (2004) 1062–1067.
[216] N. Wangfuengkanagul, O. Chailapakui, Anal. Sci. 17 (2001) 349–353.
[217] I. Christie, S. Leeds, M. Baker, F. Keedy, P. Vadgama, Anal. Chim. Acta 272 (1993) 145–150.
[218] R. Sandulescu, S. Mirel, R. Oprean, J. Pharmaceut. Biomed. Anal. 23 (2000) 77–87.
[219] R.N. Goyal, V.K. Gupta, M. Oyama, N. Bachheti, Electrochem. Commun. 7 (2005) 803–807.
[220] R.N. Goyal, S.P. Singh, Electrochim. Acta 51 (2006) 3008–3012.
[221] T.R.L.C. Paixao, E.M. Richter, J.G.A. Brito-Neto, M. Bertotti, Electrochem. Commun. 8 (2006) 9–14.
[222] P. Masawat, S. Liawruangrath, Y. Vaneesorn, B. Liawruangrath, Talanta 58 (2002) 1221–1234.
[223] R. Jiménez-Prieto, M. Silva, D. Pérez-Bendito, Analyst 122 (1997) 287–292.
[224] N. Pejic, L. Kolar-Anic, S. Anic, D. Stanisavljev, J. Pharm. Biomed. Anal. 41 (2006) 610–615.
[225] M. Alkayer, J.J. Vallon, Y. Pegon, C. Bichon, Anal. Chim. Acta 124 (1981) 113–119.
[226] M.I. Walash, A.M. Elbrashy, M.A. Sultan, Mikrochim. Acta 113 (1994) 113–124.
[227] I. Navarro, D. Gonzalez-Arjona, E. Roldan, M. Rueda, J. Pharm. Biomed. Anal. 6 (1988) 969–976.
[228] C. Wang, X. Hu, Z. Leng, G. Yang, G. Jin, Anal. Lett. 34 (2001) 2747–2759.
[229] I. Cruz Vieira, K. Omuro Lupetti, O. Fatibello-Filho, Quim. Nova 26 (2003) 39–43.
[230] O.W. Lau, S.F. Luk, Y.M. Cheung, Analyst 114 (1989) 1047–1051.
[231] Y. Ni, Y. Wang, S. Kokot, Anal. Lett. 37 (2004) 3219–3235.
[232] I. Christie, S. Leeds, M. Baker, F. Keedy, P. Vadgama, Anal. Chim. Acta 272 (1993) 145–150.
[233] A.F. Danet, V. David, I. David, Rev. Roumaine Chim. 43 (1998) 811–816.
[234] K.I. Nikolic, K.R. Velasevic, Acta Pol. Pharm. 42 (1985) 209–211.
[235] D.K. Vatsa, G.J. Rao, M. Ibrahim, M.A. Mannan, G. Karunasri, Eastern Pharma. 41 (1998) 103–104.
[236] F.A.N. El-Dien, M.A. Zayed, G.G. Mohamed, Egyptian J. Chem. 44 (2001) 51–61.
[237] A.M. Di Pietra, R. Gatti, V. Andrisano, V. Cavrini, J. Chromatogr. A 729 (1996) 355–361.
[238] A.I. Gasco-Lopez, R. Izquierdo-Hornillos, A. Jiménez, J. Chromatogr. A 775 (1997) 179–185.
[239] J.T. Franeta, D.D. Agbaba, S.M. Eric, S.P. Pavkov, S.D. Vladimirov, M.B. Aleksic, J. Pharm. Biomed. Anal. 24 (2001) 1169–1173.
[240] J.T. Franeta, D. Agbaba, S. Eric, S. Pavkov, M. Aleksic, S. Vladimirov, IL Fármaco 57 (2002) 709–713.
[241] M. Kartal, J. Pharm. Biomed. Anal. 26 (2001) 857–864.
[242] J.V. Aukunuru, U.B. Kompella, G.V. Betageri, J. Liquid Chromatogr. Rel. Technol. 23 (2000) 565–578.
[243] I.I. Hewala, Anal. Lett. 27 (1994) 561–582.
[244] J.L. Pérez, M.A. Bello, Talanta 48 (1999) 1199–1202.
[245] L. Monser, F. Darghouth, J. Pharm. Biomed. Anal. 27 (2002) 851–860.
[246] L.I. Bebawy, N.M. El-Kousy, J. Pharm. Biomed. Anal. 20 (1999) 663–670.
[247] C. Martínez-Algaba, J.M. Bermúdez-Saldaña, R.M. Villanueva-Camañas, S. Sagrado, M.J. Medina-Hernández, J. Pharm. Biomed. Anal. 40 (2006) 312–321.
[248] D. Satinsky, I. Neto, P. Solich, H. Sklenarova, M. Conceicao, B.S.M. Montenegro, A.N. Araujo, J. Sep. Sci. 27 (2004) 529–536.
[249] S. Suzen, C. Akay, S. Tartilmis, R.S. Erdol, A. Onal, S. Cevheroglu, Ankara Univ. Eczacilik Fakultesi Dergisi 27 (1998) 93–100.
[250] J.J. Bergh, A.P. Lotter, Drug Dev. Ind. Pharm. 10 (1984) 127–136.
[251] V.M. Shinde, R. Raman, Indian Drugs 35 (1998) 521–525.
[252] S.S. Zarapkar, A.A. Dhanvate, Indian Drugs 32 (1995) 405–408.
[253] A.B. Avadhanulu, A.R.R. Pantulu, Y. Anjaneyulu, Indian Drugs 31 (1994) 201–204.
[254] S.K. Pant, C.L. Jain, Indian Drugs 28 (1991) 262–265.
[255] A. Argekar, J.G. Sawant, J. Planar Chromatogr.-Modern TLC 12 (1999) 361–364.
[256] R. Thomis, E. Roets, J. Hoogmartens, J. Pharm. Sci. 73 (1984) 1830–1833.
[257] C. Akay, B. Gumusel, T. Degim, S. Tartilmis, S. Cevheroglu, Drug Metabol. Drug Interact. 15 (1999) 197–205.
[258] A. Haque, J.T. Stewart, J. Liquid Chromatogr. Relat. 22 (1999) 2159–2166.
[259] M.L. Altun, Turkish J. Chem. 26 (2002) 521–528.
[260] M.G. Mamolo, L. Vio, V. Maurich, J. Pharm. Biomed. Anal. 3 (1985) 157–164.
[261] W.R. Sisco, C.T. Rittenhouse, L.A. Everhart, A.M. McLaughlin, J. Chromatogr. 354 (1986) 355–366.
[262] J.L. Chawla, R.A. Sodhi, R.T. Sane, Indian Drugs 33 (1996) 171–178.
[263] S. Ravisankar, M. Vasudevan, M.J. Nanjan, Bijukurian, B. Suresh, Indian Drugs 34 (1997) 663–665.
[264] R.A. Sodhi, J.L. Chawla, R.T. Sane, Indian Drugs 33 (1996) 280–285.
[265] S. Ravisankar, M. Vasudevan, M. Gandhimathi, B. Suresh, Talanta 46 (1998) 1577–1581.
[266] S.K. Pant, K.M. Thomas, P.N. Gupta, B.K. Maitin, C.L. Jain, Indian J. Pharm. Sci. 52 (1990) 223–224.
[267] S.T. Patil, M. Sundaresan, I.C. Bhoir, A.M. Bhagwat, Talanta 48 (1999) 1199–1202.
[268] S.S. Zarapkar, U.P. Halkar, N.P. Bhandari, Indian Drugs 36 (1999) 710–713.
[269] J.L. Chawla, R.A. Sodhi, R.T. Sane, Indian Drugs 34 (1997) 339–345.

[270] M.J. Akhtar, S. Khan, M. Hafiz, J. Pharm. Biomed. Anal. 12 (1994) 379–382.
[271] M.A. Abuirjeie, M.E. Abdelhamid, E.S.A. Ibrahim, Anal. Lett. 22 (1989) 365–375.
[272] H. Tomankova, M. Vasatova, Pharmazie 44 (1989) 197–198.
[273] J. Krzek, M. Starek, J. Planar Chromatogr.-Modern TLC 12 (1999) 356–360.
[274] G. Indrayanto, A. Sunarto, Y. Adriani, J. Pharm. Biomed. Anal. 13 (1995) 1555–1559.
[275] B.R. Thomas, X.G. Fang, P. Shen, S. Ghodbane, J. Pharm. Biomed. Anal. 12 (1994) 85–90.
[276] N. Ramos-Martos, F. Aguirre-Gomez, A. Molina-Diaz, L.F. Capitan-Vallvey, J. AOAC Int. 84 (2001) 676–683.
[277] M.E. El-Kommos, K.M. Emara, Talanta 36 (1989) 678–679.
[278] L. Carnevale, J. Pharm. Sci. 72 (1983) 196–198.
[279] H.M. Stevens, R. Gill, J. Chromatogr. 370 (1986) 39–47.
[280] C.A. Korduba, R.F. Petruzzi, J. Pharm. Sci. 73 (1984) 117–119.
[281] D.J. Miner, P.T. Kissinger, J. Pharm. Sci. 68 (1979) 96–97.
[282] J.L. Palmer, J. Chromatogr. 382 (1986) 338–342.
[283] C.D. Kinney, J.G. Kelly, J. Chromatogr. 419 (1987) 433–437.
[284] R.M. Riggin, A.L. Schmidt, P.T. Kissinger, J. Pharm. Sci. 64 (1975) 680–683.
[285] J.W. Munson, R. Weierstall, H.B. Kostenbauder, J. Chromatogr. 145 (1978) 328–331.
[286] G.S.N. Lau, J.A.J.H. Critchley, J. Pharm. Biomed. Anal. 12 (1994) 1563–1572.
[287] M.A. Campanero, B. Calahorra, E. García-Quétglas, A. López-Ocáriz, J. Honorato, J. Pharm. Biomed. Anal. 20 (1999) 327–334.
[288] G.B. Steventon, S.C. Mitchell, R.H. Waring, Drug Metab. Drug Interact. 13 (1996) 111–117.
[289] F. Kamali, B. Herd, J. Chromatogr. 530 (1990) 222–225.
[290] S.S. Al-Obaidy, A. Li Wan Po, P.J. McKiernan, J.F.T. Glasgow, J. Millership, J. Pharm. Biomed. Anal. 13 (1995) 1033–1039.
[291] E.J. Oliveira, D.G. Watson, N.S. Morton, J. Pharm. Biomed. Anal. 29 (2002) 803–809.
[292] L.S. Jensen, J. Valentine, R.W. Milne, A.M. Evans, J. Pharm. Biomed. Anal. 34 (2004) 585–593.
[293] L.J. Brunner, S. Bai, J. Chromatogr. B 732 (1999) 323–329.
[294] B.S. Nagaralli, J. Seetharamappa, B.G. Gowda, M.B. Melwanki, J. Chromatogr. B 798 (2003) 49–54.
[295] D.S.T. Lo, T.C. Chao, S.E. Ng-Ong, Y.J. Yao, T.H. Koh, Forensic Sci. Int. 90 (1997) 205–214.
[296] C. Celma, J.A. Allué, J. Pruñonosa, C. Peraire, R. Obach, J. Chromatogr. A 870 (2000) 77–86.
[297] X. Chen, J. Huang, Z. Kong, D. Zhong, J. Chromatogr. B 817 (2005) 263–269.
[298] K.I. Burton, J.R. Everett, M.J. Newman, F.S. Pullen, D.S. Richards, A.G. Swanson, J. Pharm. Biomed. Anal. 15 (1997) 1903–1912.
[299] W. Morden, I.D. Wilson, Rapid Commun. Mass Spectrom. 10 (1996) 1951–1955.
[300] L.T. Wong, G. Solomonraj, B.H. Thomas, J. Pharm. Sci. 65 (1976) 1064–1066.
[301] L.Y. Lo, A. Bye, J. Chromatogr. 173 (1979) 198–201.
[302] B. Ameer, D.J. Greenblatt, M. Divoll, J. Chromatogr. 226 (1981) 224–230.
[303] J.N. Buskin, R.A. Upton, R.L. Williams, J. Chromatogr. 230 (1982) 443–447.
[304] D. Jung, N. Ul Zafar, J. Chromatogr. 339 (1985) 198–202.
[305] D.F. Mathis, R.D. Budd, J. Chromatogr. 439 (1988) 466–469.
[306] A. Esteban, M. Graells, J. Satorre, M. Pérez-Mateo, J. Chromatogr. 573 (1992) 121–126.
[307] R. Whelpton, K. Fernández, K.A. Wilkinson, D.R. Goldhill, Biomed. Chromatogr. 7 (1993) 90–93.
[308] W. Peng, T. Li, H. Li, E. Wang, Anal. Chim. Acta 298 (1994) 415–421.
[309] A.G. Goicoechea, M.J. Lopez de Alda, J.L. Vila-Jato, J. Liquid Chromatogr. 18 (1995) 3257–3268.
[310] H. Astier, C. Renard, V. Cheminel, O. Soares, C. Mounier, F. Peyron, J.F. Chaulet, J. Chromatogr. B 698 (1997) 217–223.
[311] K.H. Yuen, K.K. Peh, Y.L. Quah, K.L. Chan, Drug Dev. Ind. Pharm. 23 (1997) 225–228.
[312] D. Procházkova, P. Drasar, J. Vácha, Chem. Listy 91 (1997) 373–376.
[313] J.D. Baty, P.R. Robinson, J. Wharton, Biomed. Mass Spectrom. 3 (1976) 60–63.
[314] Y. Pegon, J.J. Vallon, Anal. Chim. Acta 130 (1981) 405–408.
[315] S. Murray, A.R. Boobis, J. Chromatogr. 568 (1991) 341–350.
[316] L. Steinmann, W. Thormann, Electrophoresis 17 (1996) 1348–1356.
[317] A. Kunkel, S. Günter, H. Wätzig, J. Chromatogr. A 768 (1997) 125.
[318] B.Y. Yang, J.Y. Mo, X.J. Yang, L.S. Wang, Fenxi Ceshi Xuebao 19 (2000) 13–18.
[319] F. Bohnenstengel, H.K. Kroemer, B. Sperker, J. Chromatogr. B 721 (1999) 295–299.
[320] J. Schewitz, J.C. Lindon, Analyst 123 (1998) 2835–2837.
[321] S. Heitmeier, G. Blaschke, J. Chromatogr. B 721 (1999) 93–108.
[322] L. Zhang, Q. Hu, G. Chen, Y. Fang, Anal. Chim. Acta 424 (2000) 257–262.
[323] S. Heitmeier, G. Blaschke, J. Chromatogr. B 721 (1999) 109–125.
[324] A.L. Liu, F.Y. He, Y.L. Hu, X.H. Xia, Talanta 68 (2006) 1303–1308.
[325] S. Boonkerd, M. Lauwers, M.R. Detaevernier, Y. Michotte, J. Chromatogr. A 695 (1995) 97–102.
[326] L. Suntornsuk, O. Pipitharome, P. Wilairat, J. Pharm. Biomed. Anal. 33 (2003) 441–449.
[327] S. Zhao, W. Bai, H. Yuan, D. Xiao, Anal. Chim. Acta 559 (2006) 195–199.
[328] P. Gfrorer, L.H. Tseng, E. Rapp, K. Albert, E. Bayer, Anal. Chem. 73 (2001) 3234–3239.
[329] K.D. Altria, N.W. Smith, C.H. Turnbull, J. Chromatogr. B 717 (1998) 341–353.
[330] E.P.C. Lai, E. Dabek-Zlotorzynska, Electrophoresis 20 (1999) 2366–2372.
[331] V. Pucci, R. Mandrioli, M.A. Raggi, S. Fanali, Electrophoresis 25 (2004) 615–621.
[332] M. Morder, S. Schrader, M. Winkler, P. Popp, J. Chromatogr. A 873 (2000) 95–106.
[333] W. Ahrer, E. Scherwenk, W. Buchberger, J. Chromatogr. A 910 (2001) 69–78.
[334] M.J. Hilton, K.V. Thomas, J. Chromatogr. A 1015 (2003) 129–141.