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REVIEW ARTICLE

Spectrophotometric and spectrofluorometric methods for the determination of non-steroidal anti-inflammatory drugs: A review

Ayman A. Gouda [a,*], Mohamed I. Kotb El-Sayed [b], Alaa S. Amin [c], Ragaa El Sheikh [a]

[a] *Chemistry Department, Faculty of Science, Zagazig University, Zagazig, Egypt*
[b] *Organic Chemistry Department (Pharmaceutical Biochemistry), Faculty of Pharmacy, Sana'a University, Sana'a, Yemen*
[c] *Chemistry Department, Faculty of Science, Benha University, Benha, Egypt*

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Abstract Non-steroidal anti-inflammatory drugs (NSAIDs) are the group most often used in human and veterinary medicine, since they are available without prescription for treatment of fever and minor pain. The clinical and pharmaceutical analysis of these drugs requires effective analytical procedures for quality control and pharmacodynamic and pharmacokinetic studies. An extensive survey of the literature published in various analytical and pharmaceutical chemistry related journals has been conducted and the instrumental analytical methods which were developed and used for determination of some non-steroidal anti-inflammatory, coxibs, arylalkanoic acids, 2-arylpropionic acids (profens) and *N*-arylanthranilic acids (fenamic acids) in bulk drugs, formulations and biological fluids have been reviewed. This review covers the time period from 1985 to 2010 during which 145 spectrophotometric methods including UV and derivative; visible which is based on formation of metal complexation, redox reactions, ion pair formation, charge-transfer complexation and miscellaneous; flow injection spectrophotometry as well as spectrofluorometric methods were

* Corresponding author. Tel.: +2 055 242 3346; fax: +2 055 230 8213.
E-mail address: aymangouda77@gmail.com (A.A. Gouda).

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reported. The application of these methods for the determination of NSAIDs in pharmaceutical formulations and biological samples has also been discussed.

Contents

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of drugs of diverse chemical composition and different therapeutic potentials having a minimum of three common features: identical basic pharmacological properties, similar basic mechanism of action as well as similar adverse effects. Moreover, all drugs in this group exhibit acidic character. Most NSAIDs are weak acids, with a pK_a values in the range of 3.0–5.0 (acids of medium strength).

NSAID molecules contain hydrophilic groups (carboxylic or enolic group) and lipophilic ones (aromatic ring, halogen atoms). In accordance with their acidic character, NSAIDs occur in the gastric juice in the protonated (lipophilic) form. Also in the small intestine, there are conditions favorable for absorption of weak acids. NSAID exist in highly ionized forms in plasma. Low values defining NSAIDs distribution volume

Abbreviations: NSAIDs, non-steroidal anti-inflammatory drugs; COX, cyclooxygenase; HPLC, high-performance liquid chromatography; RP-HPLC, reversed phase high-performance liquid chromatography; LC, liquid chromatography; TLC, thin layer chromatography; GC, gas chromatography; IR, infrared; AAS, atomic absorption spectrophotometry; NMR, nuclear magnetic resonance; ^{1}H NMR, proton nuclear magnetic resonance; MS, mass spectrometry; FIA, flow injection analysis; CE, capillary electrophoresis; *r*, correlation coefficient; *R*, intensity ratio; CZE, capillary zone electrophoresis; MEKC, micellar electrokinetic capillary chromatography; UV, ultraviolet; λ, wavelength; Abs, absorbance; LOD, limit of detection; LOQ, limit of quantitation; mol L^{-1}, concentration; ^{1}D, first derivative spectrophotometry; ^{1}DD, first derivative of the ratio spectra; SD, standard deviation; RSD, relative standard deviation; SPE, solid-phase extraction; $T^{1/2}$, half life time; *K*, reaction rate constant; MBTH, 3-methyl-2-benzothiazolinone hydrazone hydrochloride; ^{3}D, third-derivative spectrophotometry; PDAC, *p*-dimethylaminocinnamaldehyde; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DPPH2, 2,2-diphenyl-1-picrylhydrazine; PDAB, *p*-dimethylaminobenzaldhyde; TG, thermogravimetry; DSC, differential scanning calorimetry; TIC, 1,3,3-trimethyl-5-thiocyanato-2-[3-(1′,3′,3′-trimethyl-3′-*H*-indol-2′-ylidene)-propenyl]-indolium chloride; *p*-chloranil, tetrachloro-*p*-benzoquinone; DCNP, 2,4,dichloro-6-nitrophenol; *o*-phen, o-phenanthroline; Bipy, bipyridyl; CT, charge transfer; TCNE, tetracyanoethylene; DDQ, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone; DCNP, 2,4,dichloro-6-nitrophenol; ^{2}D, second-derivative spectrophotometry; PLS, partial least squares regression; GA-PLS, genetic algorithm-partial least squares regression; μ, ionic strength; α-CD, α-cyclodextrin; β-CD, β-cyclodextrin; ΔH, enthalpy change; ΔS, entropy change; $\Delta G°$, free energy change; IUPAC, international union of pure and applied chemistry; PHP, phenolphthalein.

(from 0.1 to 1.0) in tissues may be a proof of poor distribution of these drugs in extra vascular systems. A very high degree of binding with plasma proteins (> 97%) is the result of favorable amiliphilic properties and accounts for the fact of displacing other drugs from protein binding of NSAIDs. Most NSAIDs are metabolized in the liver by oxidation and conjugation to inactive metabolites which are typically excreted in the urine, although some drugs are partially excreted in bile. Metabolism may be abnormal in certain disease states, and accumulation may occur even with normal dosage (Starek and Krzek, 2009).

NSAIDs are classified according to their chemical structure into the following groups: salicylic acid derivatives (i.e. acetylsalicylic acid, salicylamid, sodium salicylate); aniline and *p*-aminophenol derivatives (i.e. paracetamol, phenacetyne); pyrazolone derivatives (i.e. phenylbutazone, propyphenazone); oxicams (i.e. piroxicam, meloxicam, tenoxicam, lornoxicam, droxicam); arylalkanoic acids derivatives (i.e. aceclofenac, diclofenac, etodolac, indometacin, nabumetone, sulindac, tolmetin); 2-arylpropionic acids derivatives (profens) (i.e. flurbiprofen, ibuprofen, ketoprofen, naproxen, tiaprofenic acid); *N*-arylanthranilic acids (fenamic acids) (i.e. mefenamic acid, tolfenamic acid, flufenamic acid, meclofenamic acid); enolic acid derivatives, and coxibs (i.e. celecoxib, rofecoxib, etoricoxib, parecoxib, valdecoxib); naphtylbutanone derivatives (nabumetone); sulphonamides (nimesulide); benzoxazocine derivatives (nefopam). Four groups of NSAIDs only were chosen in the present work due to more spectrophotometric and spectroflourometric methods done.

Most NSAIDs act as non-selective inhibitors of the enzyme cyclooxygenase, inhibiting both the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes. Cyclooxygenase catalyzes the formation of prostaglandins and thromboxane from arachidonic acid (itself derived from the cellular phospholipid bilayer by phospholipase A_2). Prostaglandins act (among other things) as messenger molecules in the process of inflammation.

NSAIDs are easily available and effective and thus are extensively used by patients. The growing demand for these agents stimulate a search for new even more effective drugs, but also calls for higher level of quality control of these therapeutic substances and preparations, so that they are in the highest possible degree free from any impurities that may come from the production process, as well as from decomposition products of active or auxiliary substances. Therefore, it seems appropriate to develop new analytical methods regarding their qualitative and quantitative analysis (Sherma, 2000; Rao et al., 2005; Ferenczi-Fodor et al., 2001).

The progress of analytical chemistry in the scope of instrumentalisation of the methods of chemical analysis is reflected in the use thereof in pharmacopoeia monographs as well as in the standards adopted by manufacturers. A constant place is occupied by chromatographic methods [high-performance liquid chromatography (HPLC), thin layer chromatography (TLC), and gas chromatography (GC)]. Unification of the equipment used necessitates preparation of a very accurate and detailed description of conditions for carrying out the analysis. Other meaningful methods having a big meaning are also ultraviolet–visible (UV–vis) and infrared (IR) spectrophotometry, atomic absorption spectrophotometry (AAS), nuclear magnetic resonance (NMR), mass spectrometry (MS) or spectrofluorometry. Among the analytical methods used for determining NSAIDs are also electromigrational (capillary electrophoresis (CE), capillary zone electrophoresis (CZE), and micellar electrokinetic capillary chromatography (MEKC)) and voltamperometric methods. One that has been gaining more and more applications is the flow injection analysis (FIA), whose main advantage is the full automation of the analysis, which considerably minimizes the effects of side reactions and thus increases the sensitivity and selectivity of this method.

Introduction of new methods, enabling carrying out determinations with maximum accuracy, contributes to increased interest in analytical methods as such. They should enable to simultaneously determine the individual components in multicomponent preparations and in biological material. Range of guidelines, standardizing requirements concerning the quality of drugs, have been issued. Fulfillment confirms them the appropriate quality of the product and of the analytical method used. These are numerical parameters that validate reliability of the results and enable comparing efficiency of the methods used. The process that is used to determine the above parameters is the so-called method validation (Harmonised Tripartite Guideline, 1996).

Development and validation of analytical methods are of basic importance to optimize the analysis of drugs in the pharmaceutical industry and to guarantee quality of the commercialized product. Several techniques like AAS (Khuhawar et al., 2001; Salem et al., 2000, 2001; Alpdogan and Sungur, 1999), HPLC (Hassan et al., 2008; Pavan Kumar et al., 2006; Jaiswal et al., 2007; Vinci et al., 2006; Sun et al., 2003), SPE-LC (Hirai et al., 1997), LC (Rouini et al., 2004), GC (Thomas and Foster, 2004; El Haj et al., 1999; González et al., 1996), CE (Makino et al., 2004; Ahrer et al., 2001; Pérez-Ruiz et al., 1998), potentiometric (Santini et al., 2007), conductometric (Aly and Belal, 1994) and voltammetric methods (Liu and Song, 2006) have been used for the determination of NSAIDs. Chromatographic methods have been extensively used and recommended. However, these methods generally require complex and expensive equipment, provision for use and disposal of solvents, labour-intensive sample preparation procedures and personal skills in chromatographic techniques.

Spectrophotometric and spectrofluorometric methods for the determination of drugs can be used in laboratories where modern and expensive apparatuses such as that required for GLC or HPLC are not available. However, spectrophotometric and spectrofluorometric methods are versatile and economical particularly for developing countries. Spectrophotometric and spectrofluorometric methods have several advantages such as being easy, less expensive and less time consuming compared with most of the other methods. Spectrophotometric and spectrofluorometric methods are simple and rapid; so these methods can be successfully used for pharmaceutical analysis, involving quality control of commercialized product and pharmacodynamic studies. These methods are mostly based on the formation of coloured complexes between NSAIDs and the reagent which can be determined by visible spectrophotometry. The complexes formed are mostly due to charge transfer reaction between the drug and the reagent or due to formation of ion-pair complexes. The spectrophotometric methods are simple and rapid but less sensitive. UV- and derivative spectrophotometric methods have also been widely used for NSAIDs and are covered under this review.

In the last few years, there was no review published covering all different spectrophotometric techniques like (ion pair, charge transfer, metal complexes, flow injection, derivative)

used for the determination of NSAIDs. The high importance of this class of drugs prompted us to review the most important recent spectrophotometric methods for their analysis in pure forms, in different pharmaceutical dosage forms and in biological fluids reported so far in the literature. Because of the large number of references that appeared as individual methods or as part of clinical and pharmacological studies, it is possible to make reference only to the most important papers. The present review comprises references covering the period from 1985 to 2010.

2. Spectrophotometric and spectrofluorometric methods for determination of coxibs

2.1. Celecoxib

The official method of celecoxib was potentiometric titration method with *perchloric acid* (Pharmacopoeia, 2004).

UV spectrophotometric methods have been developed for the determination of celecoxib and tizanidine hydrochloride in its pure and in its pharmaceutical formulations. Celecoxib having absorption maximum at 251.2 nm in 0.1 mol L^{-1} sodium hydroxide (Sankar, 2001). New UV spectrophotometric methods for the quantitative estimation of celecoxib, a selective COX-2 inhibitor, in pure form and in solid dosage form were developed in the present study. The linear regression equation obtained by least square regression method, was Abs = 4.949×10^{-2}. Conc. (μg mL^{-1}) + 1.110×10^{-2}. The detection limit was found to be 0.26 μg mL^{-1} (Saha et al., 2002). Ultraviolet spectrophotometric method for the determination of celecoxib in bulk and its pharmaceutical formulation (dispersible tablets and capsules) has been developed. The absorbance maxima of celecoxib in a mixture of methanol and 0.01 N sodium hydroxide (1:1 v/v) were determined at 253.1 nm. Beer's law is obeyed over concentration range of 8–22 μg mL^{-1} with correlation coefficient $r > 0.999$ (Sahu et al., 2009).

Two simple and sensitive spectrophotometric methods have been developed for the quantitative estimation of celecoxib from its capsule formulation. The first method is a UV spectrophotometric method using methanol as solvent; the drug showed absorption maximum at 253.2 nm in methanol and linearity was observed in the concentration range of 5.0–15 μg mL^{-1}. The second method is a visible spectrophotometric method, based on formation of red coloured complex of drugs with *o*-phenanthroline and ferric chloride, the complex showed absorbance maximum at 509.2 nm and linearity was observed in the concentration range of 50–400 μg mL^{-1} (Pillai and Singhvi, 2006).

A simple fluorescence method was developed for the direct determination of celecoxib in capsules. The capsules were emptied, pulverized and dissolved in either ethanol or acetonitrile, sonicated and filtered. Direct fluorescence emission was measured at 355 ± 5 nm (exciting at 272 nm). The method was fully validated and the recoveries were excellent, even in presence of excipients (Damiani et al., 2003).

2.2. Valdecoxib

The official method of valdecoxib was potentiometric titration method with *perchloric acid* (Pharmacopoeia, 2004).

Two simple, rapid, accurate and economical methods have been developed for the estimation of valdecoxib and tizanidine HCl in the mixture. Valdecoxib has an absorbance maximum at 243 nm in methanol: 0.1 mol L^{-1} HCl (1:1) mixture. The linearity was observed in the concentration range 5.0–30 μg mL^{-1}. First method is based on Q absorbance ratio and second method is based on the simultaneous equations (Sankar et al., 2007). Two methods for simultaneous estimation of valdecoxib and tizanidine in combined dosage form have been described. The first method; involves formation of Q-absorbance equation at 239.6 (isoabsorptive point) and at 241 nm, while the second method; involves formation of simultaneous equation at 241 and 229 nm, using methanol as solvent (Devarajan and Sivasubramanian, 2006). A reproducible method for simultaneous estimation of valdecoxib and paracetamol in two-component tablet formulation has been developed. The method of analysis is derivative spectroscopy to eliminate spectral interference by measuring analytical signals or $dA/d\lambda$ value at 284 nm (Aditya et al., 2006). Analytical method for the simultaneous estimation of valdecoxib and paracetamol in combined tablet dosage form by Vierodt's UV spectrophotometric method was validated. The λ_{max} value of valdecoxib in 0.1 mol L^{-1} NaOH was 244 nm. Beer's law is valid in the concentration range of 1.0–6.0 μg mL^{-1}. The A1% 1 cm values for valdecoxib at 244 nm were 520 and 420 (Nagulwar et al., 2006). UV spectrophotometric method has been developed for the simultaneous estimation of valdecoxib and tizanidine in pharmaceutical dosage form. The proposed method is based on the Vierodt's simultaneous equations. Valdecoxib absorption maxima at 239.0 nm in methanol. The linearity was observed in the concentration range of 2–18 μg mL^{-1} (Sharma et al., 2009). UV method was used for bulk form as well as the formulation of the valdecoxib and was expanded to study the dissolution profile of valdecoxib tablets. The measurements were done at 241 nm, linear concentration range was observed to be 3–17 μg mL^{-1}. The percentage recovery was found to be between 99.52 and 100.32 (Baviskar et al., 2009).

A spectrophotometric method has been developed for the determination of valdecoxib in pure and pharmaceutical dosage forms. The method is based on the reaction of valdecoxib with potassium permanganate to form a bluish green coloured chromogen with an absorption maximum at 610 nm. Beer's law was obeyed in the range of 5.0–25 μg mL^{-1}. The molar absorpitivity is 7.1437×10^{-3} L mol^{-1} cm^{-1} (Suganthi et al., 2006).

2.3. Rofecoxib

The official method of rofecoxib was potentiometric titration method with *perchloric acid* (Pharmacopoeia, 2004).

Two different UV spectrophotometric methods were developed for the determination of rofecoxib in bulk form and in pharmaceutical formulations. The first method, a UV spectrophotometric procedure, was based on the linear relationship between the rofecoxib concentration and the λ_{max} amplitude at 279 nm. The second one, the first derivative spectrophotometry, was based on the linear relationship between the rofecoxib concentration and the first derivative amplitude at 228, 256 and 308 nm. Calibration curves were linear in the concentration range using peak to zero 1.5–35 μg mL^{-1} (Erk and Altuntas,

2004). Rofecoxib has been determined in the presence of its photo-degradation product using first derivative spectrophotometry (^{1}D) and first derivative of the ratio spectra (^{1}DD) by measuring the amplitude at 316.3 and 284 nm for ^{1}D and ^{1}DD, respectively. Rofecoxib can be determined in the presence of up to 70% and 80% of the photo-degradation product by the ^{1}D and ^{1}DD, respectively. The linearity range of both the methods was the same (5.8–26.2 μg mL^{-1}) with mean percentage recovery of 100.08 ± 0.84 and 100.06 ± 1.06 for ^{1}D and ^{1}DD, respectively. ^{1}D method was used to study kinetics of rofecoxib photo-degradation that was found to follow a first-order reaction. The $T^{1/2}$ was 20.2 min while K (reaction rate constant) was 0.0336 mol min^{-1} (Shehata et al., 2004). Rofecoxib was assayed by UV spectrophotometry, the concentration ranges were 2.0–30 μg mL^{-1} (Duran et al., 2004). UV and visible spectrophotometric methods have been developed for the determination of rofecoxib in pure and its pharmaceutical formulations. In UV method rofecoxib solution in methanol medium showed absorption maximum at 285 nm, whereas in visible spectrophotometric method it reacts with ferric chloride and 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) reagent and forms a green coloured chromogen having absorption maximum at 625 nm (Reddy et al., 2002).

A spectrofluorometric method was described to determine rofecoxib at very low concentrations (25–540 ng mL^{-1}) where rofecoxib is converted to its photo-degradate product, which possesses a native fluorescence that could be measured (Shehata et al., 2004).

2.4. Etoricoxib

The official method of etoricoxib was potentiometric titration method with *perchloric acid* (Pharmacopoeia, 2004).

A presented method was performed at 284 nm for the analysis of etoricoxib formulations. Extraction of etoricoxib from tablet was carried out using methanol. The linearity range was 5.0–35 μg mL^{-1} (Shakya and Khalaf, 2007). A simple, rapid, precise spectrophotometric method for estimation of etoricoxib in bulk drug, dosage forms and human plasma was developed. Sample preparation for the developed method employs 90% methanolic sodium hydroxide (0.1 mol L^{-1}) as the solvent system for analyzing bulk drug and dosage forms, while precipitation using acetonitrile (direct procedure) and liquid–liquid extraction with ethyl acetate (indirect procedure) was utilized for its determination in human plasma samples. All samples were analyzed spectrophotometrically at 280 nm. For analysis of dosage forms, the method was found to be linear in the range of 3.0–60 μg mL^{-1} (r^2 = 0.9997 and 0.9998); for estimation of human plasma samples, the method was found to be linear in the range of 0.1–20 μg mL^{-1} (r^2 = 0.9998 and 0.9994, respectively, for direct and indirect method) (Vadnerkar et al., 2006). Sensitive UV spectrophotometric methods for the determination of etoricoxib and ezetimibe were having absorption maximum at 235 and 230 nm, respectively, and these methods were extended to pharmaceutical preparations (Sankar et al., 2005). Extractive spectrophotometric methods for the determination of etoricoxib in tablets through ion–association complexes with bromocresol

Table 1 Comparison between the spectrophotometric methods for determination of coxibs.

Drug	Method	λ_{max} (nm)	Linear range (μg mL^{-1})	Ref.
Celecoxib	UV methods	251.2	–	Sankar (2001)
		–	–	Saha et al. (2002)
		253.1	8–22	Sahu et al. (2009)
		253.2	5.0–15	Pillai and Singhvi (2006)
	1,10-Phenanthroline/ferric chloride	509.2	50–400	Pillai and Singhvi (2006)
	Spectrofluorometric method	λ_{em} = 355 ± 5 λ_{ex} = 272	–	Damiani et al. (2003)
Valdecoxib	UV methods	243	5.0–30	Sankar et al. (2007)
		239.6 241	–	Devarajan and Sivasubramanian (2006)
		284	–	Aditya et al. (2006)
		244	1.0–6.0	Nagulwar et al. (2006)
		239	2–18	Sharma et al. (2009)
		241	3–17	Baviskar et al. (2009)
	Potassium permanganate	610	5.0–25	Suganthi et al. (2006)
Rofecoxib	UV methods	279, 228, 256 and 308	1.5–35	Erk and Altuntas (2004)
		316.3 284	5.8–26.2	Shehata et al. (2004)
		–	2.0–30	Duran et al. (2004)
		285	–	Reddy et al. (2002)
	Ferric chloride/(MBTH)	625	–	Reddy et al. (2002)
	Spectrofluorometric method	–	25–540 ng/ml	Shehata et al. (2004)
Etoricoxib	UV methods	284	5.0–35	Shakya and Khalaf (2007)
		280	3.0–60	Vadnerkar et al. (2006)
		235	–	Sankar et al. (2005)
	BCG	416	–	Shah et al. (2009)
	BCP	408	–	

green (BCG) and bromocresol purple (BCP) were soluble in chloroform. The complex of etoricoxib with BCG and BCP showed λ_{max} at 416 and 408 nm, respectively. Molar absorptivity, 1.9331×10^4 and 1.6642×10^4 L mol^{-1} cm^{-1} for BCG and BCP, respectively (Shah et al., 2009).

Table 1 shows comparison between the published spectrophotometric and spectrofluorometric methods for coxibs.

3. Spectrophotometric and spectrofluorometric methods for determination of arylalkanoic acids

3.1. Aceclofenac

The official method of aceclofenac was potentiometric titration method with *sodium hydroxide* (Pharmacopoeia, 2004).

Four procedures for simultaneous estimation of paracetamol and aceclofenac in tablet dosage form have been developed. The first method employs the formation and solving of simultaneous equation using 273 nm as the wavelength for forming equation. The second method employs first order derivative spectroscopy to eliminate spectral interference. The third method employs selection of area under curve in wavelength region of 271–275 nm and solving the equation. The fourth method employed 266 nm as λ_1 (isobestic point) and 244 nm as λ_2, which is the λ_{max} of paracetamol. The solution of drug in methanol obeys Beer's law in the concentration range 10–100 μg mL^{-1} for aceclofenac (Nikam et al., 2007). A simultaneous equation and *Q*-analysis UV spectrophotometric method has been developed for the simultaneous determination of aceclofenac and paracetamol from the combined tablet dosage form. The method involves solving of simultaneous equation value analysis based on measurement of absorptivity at 276, 249 and 270 nm, respectively. Linearity was in the range 2.0–25 μg mL^{-1} for aceclofenac (Jain et al., 2007). Three accurate methods; multicomponent, two wavelength and simultaneous equations using area under curve have been described for the simultaneous estimation of aceclofenac and paracetamol in tablet dosage form. Absorption maxima of aceclofenac in methanol diluted with glass double distilled water was found to be 274.5 nm. Beer's law was obeyed in the concentration range 2.0–20 μg mL^{-1} for aceclofenac (Mahaparale et al., 2007). A derivative spectrophotometric procedure has been developed for the simultaneous determination of individual combination of aceclofenac and tramadol with paracetamol in combined tablet preparations. Tablet extracts of the drugs were prepared in distilled water. The zero crossing point technique and the compensation technique were used to estimate the amount of each drug in the combined formulations, and were compared. Calibration graphs are linear ($r = 0.9999$), with a zero intercept up to 24 μg mL^{-1} of each drug in combination with paracetamol. Detection limits at the $p = 0.05$ level of significance were calculated to be 0.5 μg mL^{-1} (Srinivasan et al., 2007). An UV-spectrophotometric method was developed for the estimation of aceclofenac in tablets. In this method, aceclofenac is determined accurately having absorbance maximum at 203 nm. Beer's law is obeyed in the concentration range 0.0–20 μg mL^{-1} (Saravanan et al., 2006). A spectrophotometric method for the determination of aceclofenac in its pharmaceutical dosage forms has been developed. Aceclofenac shows absorption maximum at 273.5 nm and obeyed Beer–Lambert's law in the concentration range of 5.0–45 μg mL^{-1} in the 7.4 phosphate buffer (Dashora et al., 2006). Two spectrophotometric methods for the determination of aceclofenac and paracetamol in tablets have been developed. First method is based on the additivity of absorbances. Second method is based on the determination of graphical absorbance ratio at two selected wavelengths; one being the isoabsorptive point for the drug (230 nm). Beer–Lambert's law is obeyed in the concentration range 1.0–10 μg mL^{-1} (Mishra and Garg, 2006). New methods for the determination of aceclofenac in the presence of its degradation product (diclofenac) were described. Method A utilizes third derivative spectrophotometry at 242 nm. Method B is a ^{1}DD spectrophotometric method based on the simultaneous use of the first derivative of ratio spectra and measurement at 245 nm. Method C is a pH-induced difference (ΔA) spectrophotometry using UV measurement at 273 nm. Regression analysis of a Beer's plot showed good correlation in the concentration ranges 5.0–40, 10–40, 15–50 μg mL^{-1} for methods A, B and C, respectively (Hasan et al., 2003). Three methods were developed for the determination of aceclofenac in the presence of its degradation product, diclofenac. In the first method, third-derivative spectrophotometry (^{3}D) is used. The ^{3}D absorbance is measured at 283 nm where its hydrolytic degradation product diclofenac does not interfere. The suggested method shows a linear relationship in the range of 4.0–24 μg mL^{-1} with mean percentage accuracy of 100.05 ± 0.88. This method determines the intact drug in the presence of up to 70% degradation product with mean percentage recovery of 100.42 ± 0.94. The second method depends on ratio-spectra first-derivative (RSD_1) spectrophotometry at 252 nm for aceclofenac and at 248 nm for determination of the degradation product over concentration ranges of 4.0–32 μg mL^{-1} for both aceclofenac and diclofenac with mean percentage accuracy of 99.81 ± 0.84 and 100.19 ± 0.72 for pure drugs and 100.17 ± 0.94 and 99.73 ± 0.74 for laboratory-prepared mixtures, respectively (El-Saharty et al., 2002).

Two methods have been developed for the quantitative estimation of aceclofenac from tablet formulation using Folin–Ciocalteu reagent. Aceclofenac forms a blue coloured chromogen with the reagent, which shows absorbance maxima at 642.6 nm and linearity in the concentration range of 80–160 μg mL^{-1} of drug (Singhvi and Goyal, 2007). Two convenient visible spectrophotometric methods have been developed for the estimation of aceclofenac in tablet formulation. The developed methods are based on the formation of chloroform extractable complex of aceclofenac with orange G in acidic medium and naphthol green in aqueous medium. The extracted complex with orange G shows absorbance maxima at 481 nm and linearity in the concentration range of 10–80 μg mL^{-1}. The extracted complex with naphthol green shows absorbance maxima at 633.6 nm and linearity in the concentration range of 0.2–1.0 μg mL^{-1} (Goyal and Singhvi, 2006). A spectrophotometric method for the determination of aceclofenac in its pharmaceutical dosage forms has been developed. The method is based on the formation of a coloured complex of the drug with ferric nitrate in acidic medium, which has absorption maximum at 470 nm. Beer's law is obeyed over concentration range of 75–200 μg mL^{-1} (Mishra and Garg, 2006). Quantitative determination of aceclofenac in pure form and in pharmaceutical formulation was presented. The method is based on the reaction between the drug via its secondary aromatic amino group and *p*-dimethylamino-

cinnamaldehyde (PDAC) in acidified methanol to give a stable coloured complex after heating at 75 °C for 20 min. Absorption measurements were carried out at 665.5 nm. Beer's law is obeyed over concentration range 20–100 μg mL^{-1} with mean recovery 100.33 ± 0.84 (Zawilla et al., 2002). A spectrophotometric procedure for the assay of aceclofenac has been developed. The method is based on the reaction of aceclofenac with 2,2-diphenyl-1-picrylhydrazyl (DPPH). The latter is employed to abstract a hydrogen atom from the drug thereby promoting a process of radical coupling. This results in a reduction of the violet colour of DPPH with the formation of the yellow coloured 2,2-diphenyl-1-picrylhydrazine (DPPH2). The decrease in the intensity of the violet colour is used to measure the concentration of the drug. All measurements are made at λ = 520 nm on methanolic solutions of the reagent and drugs. Beer's law is obeyed in the range of 5.0–30 μg mL^{-1} (Salem, 2000). A spectrophotometric method was adopted for the analysis of the anti-inflammatory drug, aceclofenac. The method is based on the formation of coloured complexes between the drug and *p*-dimethylaminobenzaldehyde reagent (PDAB) in the presence of sulfuric acid and ferric chloride. Measurement of the absorbance was carried out at 545.5 nm. Regression analysis of Beer's plots showed good correlation in the concentration ranges 8.0–55 μg mL^{-1}. The spectrofluorometric method in samples of aceclofenac in the phosphate buffer pH 8.0 showed native fluorescence at λ = 355 nm when excitation was at 250 nm. The calibration graph was rectilinear from 2.0 to 8.0 μg mL^{-1}. The proposed methods are applied successfully for the determination of the drug in bulk powder with a mean accuracy of 100.03 ± 0.38 in the PDAB method and of 99.88 ± 0.45 in the spectrofluorometric method (El Kousy, 1999).

3.2. Diclofenac

The official method of diclofenac was potentiometric titration method with *perchloric acid* (Pharmacopoeia, 2004).

Spectrophotometric methods were developed and validated for quantitation of diclofenac potassium and tizanidine in tablet dosage form. Three new analytical methods were developed based on the simultaneous estimation of drugs in a binary mixture without previous separation. In multiwavelength technique, the binary mixture was determined by mixed standards and three sampling wavelengths of 277, 295 (isobestic point), and 320 nm. In the simultaneous equation method, the drugs were determined by using the absorptivity values of diclofenac potassium at selected wavelength, viz., 277 nm. The standard deviation value for the validation parameters was found to be between 0.08% for multiwavelength technique and between 0.069% for simultaneous equation method. The graphical absorbance ratio method was performed by absorbances at 277, 295 (isobestic point), and 320.4 nm of their mixture (Sanjay et al., 2006). Spectrophotometric methodology was applied in order to determine benzyl alcohol and diclofenac in injectable formulations by applying a multivariate calibration method. By a multivariate calibration method such as partial least squares, it is possible to obtain a model adjusted to the concentration values of the mixtures used in the calibration range. In this study, the concentration model is based on absorption spectra in the 230–320 nm range for 25 different mixtures of benzyl alcohol and diclofenac. Calibration matrix contains 1.0–50 μg mL^{-1} for diclofenac (Ghasemi et al., 2005a). Two spectrophotometric methods were presented for simultaneous quantitative determination of benzyl alcohol and diclofenac in various pharmaceutical forms. The first method makes use of a derivative of the double-divisor-ratio spectrum of optical density. The linear determination range is 12–45 μg mL^{-1}. In the second method, the analytical signals are measured at wavelengths corresponding to either maxima or minima for both drugs in the spectra of the first derivative of the ratio of optical densities of the sample and the standard solution of one of the drugs. In this case, the linear determination ranges is 14–45 μg mL^{-1} (Ghasemi et al., 2005b). A procedure for determination of diclofenac in the presence of B vitamins was described, based on UV measurements and partial least squares. The interference of thiamine and pyridoxine were modeled using an experimental design constructed in the ranges of 10–50 μmol L^{-1} for diclofenac (Sena et al., 2004). Spectrophotometric methodology was used in order to determine diclofenac and benzyl alcohol in injectable formulations by applying, on the one hand, the first-derivative method of crossing zero for diclofenac sodium and on the other, the second derivative for benzyl alcohol (De Micalizzi et al., 1998). Two methods for the determination of the diclofenac salts [sodium or diethylammonium] in three pharmaceutical formulations (tablets, suppositories and gel) are presented. In the first, diclofenac salt is determined both by measuring the absorbance of the solutions at a fixed wavelength (λ = 276 nm) and using a multiwavelength computational program to process the spectrophotometric data in a selected range (λ = 230–340 nm). In this case, the analysis is performed measuring the peak-to-peak amplitude in the first-derivative UV spectrum (^{1}D 261.296). In the second method, diclofenac is precipitated in acid medium and determined by the analysis of the endothermic peak (t_p = 182 °C) in the DSC curve obtained in nitrogen atmosphere. Finally, some aspects of chemical (solubility, acid–base equilibria, redox reaction), spectroscopic (UV, IR) and thermoanalytical (TG, DSC) behaviour of DS and DH and the values of the parameters which enable to calculate the UV spectrum of DS in aqueous solution are reported (Bucci et al., 1998). A second derivative spectrophotometric method (^{2}D) has been developed for the determination of the degradation products from diclofenac sodium in gel-ointment. The amplitudes in the second derivative spectra at 260 and 265 nm were selected to determine oxindol. The LOD of oxindol was estimated to be 0.01% with respect to the gel-ointment (Karamancheva et al., 1998). Three procedures for simultaneous estimation of diclofenac sodium and paracetamol in two component tablet formulation have been developed. The methods employ first derivative ultraviolet spectrophotometry, simultaneous equations and the program in the multicomponent mode of analysis of the instrument used, for the simultaneous estimation of the two drugs. In 0.02 mol L^{-1} sodium hydroxide, diclofenac sodium has maxima at 276 nm (Bhatia et al., 1996). A procedure for simultaneous estimation of diclofenac sodium, chlorzoxazone and paracetamol in three component tablet formulations has been developed. The method is based on the native ultraviolet absorbance maxima of the three drugs in 0.02 mol L^{-1} sodium hydroxide. Diclofenac sodium has absorbance maxima at 276 nm (Bhatia and Dhaneshwar, 1995). Spectrophotometric methods for simultaneous estimation of diclofenac sodium and rabeprazole in combined dosage form. Methanol was used as a common solvent for both the drugs. Linearity was

observed at both wavelengths in the concentration range of 10–50 μg mL^{-1} for each drug (Choudhary et al., 2010).

A new spectrophotometric method has been developed for the determination of diclofenac sodium in pharmaceutical preparations. This method is based on the reaction of diclofenac sodium with an analytical reagent 1,3,3-trimethyl-5-thiocyanato-2-[3-(1′,3′,3′-trimethyl-3′-*H*-indol-2′-ylidene)-propenyl]-indolium chloride (TIC) at pH 8.0–11.0 and the extraction of ion associate coloured complex. This ion associate complex (1:1) was detected and extracted with toluene and an absorption maximum at 566.2 nm against a blank reagent. The calibration graph was linear from 0.9 to 11 μg mL^{-1} of diclofenac and the LOD was 0.86 μg mL^{-1} (Kormosh et al., 2008). An extractive-spectrophotometric method for the preconcentration and determination of diclofenac was developed. In a strong nitric acid medium, diclofenac produced a yellowish compound in a water/tetrahydrofuran/perfluorooctanoic acid homogeneous phase that could be extracted into a sedimented microdroplet. The concentration of the extracted coloured compound in the microdroplet was determined by measuring its absorbance at 376 nm. The maximum absorbance was achieved in 1.5 and 7.0 mol L^{-1} aqueous and methanolic solutions of nitric acid. The absorbance of diclofenac solutions in water and methanol obeyed Beer's law, over the range of 1.0–30 and 0.5–40 μg mL^{-1}, with molar absorptivities of 7.4×10^3 and 1.3×10^4 L mol^{-1} cm^{-1}, respectively. The LOD achieved with the proposed method was 0.03 ng mL^{-1} (Ghiasvand et al., 2008). A kinetic method based on a ligand-exchange reaction for the determination of micro quantities of diclofenac sodium was described. The reaction was followed spectrophotometrically by monitoring the rate of appearance of the cobalt diclofenac complex at 376 nm. The optimized conditions yielded a theoretical LOD of 1.29 μg mL^{-1} based on the $3S_b$ criterion (Mitić et al., 2007). An effective method for the determination of sodium or potassium diclofenac is proposed in its pure form and in their pharmaceutical preparations. The method is based on the reaction between diclofenac and tetrachloro-*p*-benzoquinone (*p*-chloranil), in methanol medium. This reaction was accelerated by irradiating of reactional mixture with microwave energy (1100 W) during 27 s, producing a charge transfer complex with a maximum absorption at 535 nm. Beer's law is obeyed in a concentration range from of 1.25×10^{-4} to 2.00×10^{-3} mol L^{-1} with a correlation coefficient of 0.9993 and molar absorptivity of 0.49×10^3 L mol^{-1} cm^{-1}. The LOD was 1.35×10^{-5} mol L^{-1} and the LOQ was 4.49×10^{-5} mol L^{-1} (Ciapina et al., 2005). A spectrophotometric method was proposed for determination of sodium diclofenac in pharmaceutical preparations based on its reaction with concentrated nitric acid (63% w/v). The reaction product is a yellowish compound with maximum absorbance at 380 nm. The corresponding calibration curve is linear over the range of 1.0–30 μg L^{-1}, while the LOD is 0.46 μg L^{-1} (Matin et al., 2005). A modified procedure for the visible spectrophotometric determination of diclofenac, in pharmaceutical preparations using as reagent an aqueous solution of copper(II), is proposed. A green colour complex is formed between copper(II) and diclofenac with a maximum light absorption at 680 nm. The optimal conditions were found to be 5.3 (pH of the solution to be extracted), 50.0 mg mL^{-1} (copper(II) acetate in 0.01 mol L^{-1} acetic acid solution) and three extractions with chloroform using a total volume of 5.0 mL. The intrinsic RSD of the proposed method was about 2.3% for sodium diclofenac and 2.7% for potassium diclofenac. The linear correlation coefficient, *r*, was 0.9984 for sodium diclofenac salt and 0.9993 for potassium diclofenac salt. The linear range goes from 1.0 to 25.0 mg mL^{-1} in the working solution. The LOD is 0.2 mg mL^{-1} and the LOQ is 0.7 mg mL^{-1} (De Souza and Tubino, 2005). A spectrophotometric method for the determination of diclofenac sodium in pure form and in pharmaceutical formulations was developed. The method is based on the oxidation of diclofenac sodium by iron(II) in the presence of *o*-phenanthroline. The formation of tris(*o*-phenanthroline) iron(II) complex (ferroin) upon the reaction of diclofenac sodium with an iron(III)-*o*-phenanthroline mixture in acetate buffer solution of pH 4.4, respectively, was investigated. The ferroin complex is measured at 510 nm against a reagent blank prepared in the same manner. The optimum experimental parameters for the colour production are selected. Beer's law is valid within a concentration range of 1.0–32 μg mL^{-1}. For more accurate results, Ringbom optimum concentration ranges are 2.0–30 μg mL^{-1}. The molar absorptivity is 1.15×10^4 L mol^{-1} cm^{-1}, whereas Sandell sensitivity is 2.78 ng cm^{-2}. The method gave a mean percentage recoveries 99.8 ± 1.2% (El-Didamony and Amin, 2004). Spectrophotometric determination of diclofenac sodium using 2,2-diphenyl-1-picrylhydrazyl (Salem, 2000) was investigated.

Simple spectrophotometric methods are described (Agrawal and Shivramchandra, 1991) for the determination of diclofenac. In the first method diclofenac reduces iron(III) to iron(II) when heated in aqueous solution. The ferrous ions produced react with 2,2′-bipyridine to form a complex having a maximum absorbance at 520 nm. The reaction obeys Beer's law for concentrations of 10–80 μg mL^{-1}. In the second method, diclofenac is treated with methylene blue in the presence of phosphate buffer (pH 6.8) and the complex is extracted with chloroform. The complex has a maximum absorbance at 640 nm and the graph of absorbance against concentration is linear in the range 5–40 μg mL^{-1}. A multifactor optimization technique was successfully applied to develop a new spectrophotometric method in which diclofenac sodium is analyzed and determined as it is Fe(III) complex. The effect of simultaneously varying the pH, ionic strength and concentration of colour reagents in the reaction mixture were studied. A four-variable two-level factorial design was used to investigate the significance of each variable and interactions between them. A response surface design was used to optimize complex formation and extraction. It was established that diclofenac reacts with Fe(III) chloride, in the presence of ammonium thiocyanate, in the pH range 4.2–6.5, forming a red chloroform extractable (2:1) complex with maximum absorbance at 481 nm. By applying the methods of Sommer and Job involving non-equimolar solutions the conditional stability constant of the complex, at the optimum pH of 6.0 and an ionic strength μ = 0.19 mol L^{-1}, was found to be $10^{6.4}$. Good agreement with Beer's law was found for diclofenac concentrations up to 1.57–15.7 mmol L^{-1} (0.1–1.0 mg mL^{-1}). The nominal percent recovery of diclofenac was 98.8% (n = 10). The lower limit of sensitivity of the method was found to be 14.7 μg mL^{-1} (Agatonovic-Kuštrin et al., 1997). A colorimetric method for the quantitative determination of diclofenac sodium in pure form and in pharmaceutical preparations was developed. It was based on the interaction of the secondary aromatic amine with *p*-dimethylaminocinnamaldehyde in acidified absolute methanol medium to form very stable red [λ_{max}

at 538 nm] products. Beer's law was obeyed over the range 10–80 μg mL^{-1}. The reactants were heated on a boiling water bath for 6.0 min (El Sherif et al., 1997). A validated method has been developed for estimation of diclofenac diethylammonium in bulk and formulation. The present method utilizes the reaction of diclofenac diethylammonium with 1.0% w/v potassium ferricyanide in presence of 0.5% w/v sodium hydroxide which produces orange chromogen with maximum absorbance at 450 nm and obeys Beer's law in the concentration range of 2.0–12 μg mL^{-1}. The chromogen is stable for more than 30 min (Validya and Parab, 1995). A spectrophotometric method was described for the determination of diclofenac sodium in bulk samples and pharmaceutical preparations. The method is based on the reaction of diclofenac sodium with *p*-*N*,*N*-dimethylphenylenediamine in the presence of $S_2O_8^{2-}$ or Cr(VI) whereby an intensely coloured product having maximum absorbance at 670 nm is developed. The reaction is sensitive enough to permit the determination of 2.0–24 μg mL^{-1} (Sastry et al., 1989).

Two FI spectrophotometric methods were proposed for the determination of diclofenac in bulk samples and pharmaceuticals. Both methods are based on the reaction of diclofenac with potassium ferricyanide in a sodium hydroxide medium. The absorbance of the orange products obtained is measured at 455 nm. The corresponding calibration graphs are linear over the range 0.20–20 μg mL^{-1}, while the LOD were 0.05 μg mL^{-1} (García et al., 2001). A flow-through sensor for the determination of diclofenac sodium was developed, based on retention of the analyte on a Sephadex QAE A-25 anion-exchange resin packed in a flow-cell of 1.0 mm of optical path length, and monitoring of its intrinsic absorbance by UV-spectrophotometry at 281 nm. Diclofenac could be determined in the concentration ranges 2.0–40.0, 1.0–22.0 and 0.5–14.0 μg mL^{-1} with RSD (%) ranging from 1.05 to 1.53 for sample volumes of 300, 600 and 1200 μL, respectively. The proposed sensor was satisfactorily applied to the rapid determination of diclofenac in commercial pharmaceutical preparations and in semi-synthetic pharmaceuticals containing diclofenac and paracetamol (Ortega-Barrales et al., 1999). A FI spectrophotometric method for the determination of diclofenac sodium based on the formation of coloured compound with Ce(IV)–3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in 3.0×10^{-2} mol L^{-1} H_2SO_4 medium was proposed. Using the peak height as a quantitative parameter diclofenac was determined at 580 nm over the range 0.2–8.0 μg mL^{-1}. The method was successfully applied to the determination of diclofenac in pharmaceuticals and urine samples (García et al., 1998). Diclofenac sodium, famotidine and ketorolac tromethamine were determined by FIA with spectrophotometric detection. The sample solution 5.0–50 μg mL^{-1} of diclofenac sodium, in methanol was injected into a flow system containing 0.01% (w/v) of 2,4,dichloro-6-nitrophenol (DCNP) in methanol. The colour produced due to the formation of a charge transfer complex was measured with a spectrophotometric detector set at 450 nm. A sampling rate of 40 per hour was achieved with high reproducibility of measurements (RSD $\leqslant$ 1.6%) Kamath et al., 1994.

The spectrophotometric determination of trace amounts of diclofenac was carried out by liquid–liquid extraction using acridine yellow with a flow system (Pérez-Ruiz et al., 1997). The determination of diclofenac sodium in the range of 3.0–80 μg mL^{-1} was possible with a sampling frequency of 40 samples h^{-1}. The spectrofluorometric determination of diclofenac [2-(2,6-dichloroanilino)-phenylacetic acid] in pharmaceutical tablets and ointments was described (Damiani et al., 1999). It involves excitation at 287 nm of an acid solution (HCl 0.01 M) of the drug and measurement of the fluorescence intensity at 362 nm. The linear range is 0.2–5.0 μg mL^{-1}. No interference is observed from the excipients or from other drugs which accompany diclofenac in certain formulations (paracetamol or cianocobalamine).

The next study focuses on the complex formed between α-cyclodextrin (α-CD) and diclofenac in aqueous solution and also on its potential analytical applications. It was corroborated that the fluorescence emission band of diclofenac is significantly intensified in the presence of α-CD. From the changes in the fluorescence spectra, it was concluded that α-CD forms a 1:1 inclusional complex with diclofenac and its equilibrium constant was calculated to be $1.20(3) \times 10^3$ mol L^{-1}. With the purpose of characterizing the inclusion complex, the acid–base behaviour of diclofenac in both the presence and absence of α-CD was spectrophotometrically investigated. From the results obtained, it was inferred that both the carboxyl and the secondary amino groups of the guest molecule remain outside the cyclodextrin cavity. Further details on the complex structure were obtained by ^1H NMR measurements and semiempirical calculations. In addition to the analysis of the α-CD-diclofenac interaction, a new approach for the quantification of diclofenac in the presence of α-CD is described in the range 0.0–5.0 μg mL^{-1} (Arancibia et al., 2000). A spectrofluorometric method for the microdetermination of diclofenac sodium has been developed through its reaction with cerium(IV) in an acidic solution and measurement of the fluorescence of the Ce(III) ions produced. Under the optimum experimental conditions for the oxidation reaction, 1.0 mol L^{-1} H_2SO_4 with 90 min of heating time (100 °C), the range of application is 124.3–600 ng mL^{-1} and the limit of detection is 72.7 ng mL^{-1} (Castillo and Bruzzone, 2006). A new method has been devised for the determination of diclofenac sodium in bulk and in pharmaceutical preparations using Eu(III) ions as the fluorescent probe. The technique was built around the hypersensitive property of the transitions of the fluorescent probe ion, Eu(III), at 616 nm. This is normally a forbidden transition, but the interaction with diclofenac sodium, which contains a carboxylic group, makes the transition allowed and enhances the intensity of its fluorescence emission. The Eu(III) fluorescence emission at 592 nm comes from a non-hypersensitive transition and is not affected by ligation. The intensity ratio, R, defined as I_{592}/I_{616}, was used as a measure of the percentage of bound probe ions. Diclofenac and Eu(III) forms a (1:1) molar complex. The relative stability constant of the complex was found to be 10^5. A linear relationship between bound Eu(III) and the concentration of diclofenac sodium was found for concentrations from 10 to 200 μg mL^{-1}, with a recovery percentage of 100.22 ± 2.27 (Carreira et al., 1995).

3.3. Etodolac

The official method of etodolac was potentiometric titration method with *tetrabutylammonium hydroxide* (Pharmacopoeia, 2004).

Two spectrophotometric and spectrofluorometric methods were adopted for the analysis of the anti-inflammatory drugs,

Table 2 Comparison between the spectrophotometric methods for determination of arylalkanoic acids.

Name of drug	Method	λ_{max} (nm)	Linear range ($\mu g\ mL^{-1}$)	Ref.
Aceclofenac	UV methods	273	10–100	Nikam et al. (2007)
		276, 249 and 270	2.0–25	Jain et al. (2007)
		274.5	2.0–20	Mahaparale et al. (2007)
			up to 24	Srinivasan et al. (2007)
		203	0.0–20	Saravanan et al. (2006)
		273.5	5.0–45	Dashora et al. (2006)
		230	1.0–10	Mishra and Garg (2006)
		242	5.0–40	Hasan et al. (2003)
		245	10–40	
		273	15–50	
		283	4.0–24	El-Saharty et al. (2002)
		252	4.0–32	
	Folin–Ciocalteu	642.6	80–160	Singhvi and Goyal (2007)
	Orange G in acidic medium	481	10–80	Goyal and Singhvi (2006)
	Naphthol green in aqueous medium	633.6	0.2–1.0	
	Ferric nitrate in acidic medium	470	75–200	Mishra and Garg (2006)
	p-Dimethylaminocinnamaldehyde (PDAC)	665.5	20–100	Zawilla et al. (2002)
	2,2-Diphenyl-1-picrylhydrazyl (DPPH)	520	5.0–30	Salem (2000)
	p-Dimethylaminobenzaldhyde reagent (PDAB)/sulfuric acid/ferric chloride	545.5	8.0–55	El Kousy (1999)
	Spectrofluorimetric method	λ_{em} = 355 λ_{ex} = 250	2.0–8.0	El Kousy (1999)
Diclofenac	UV methods	277, 295 (isobestic point), and 320		Sanjay et al. (2006)
		277		
		277, 295 and 320.4		
		230–320	1.0–50	Ghasemi et al. (2005a)
			12–45	Ghasemi et al. (2005b)
			14–45	
			10–50 $\mu mol\ L^{-1}$	Sena et al. (2004)
		276		Bucci et al. (1998)
		260 and 265		Karamancheva et al. (1998)
		276		Bhatia et al. (1996)
	3,3-Trimethyl-5-thiocyanato-2-[3-(1′,3′,3′-trimethyl-3′-*H*-indol-2′-ylidene)-propenyl]-indolium chloride (TIC)	566.2	0.9–11	Kormosh et al. (2008)
	Nitric acid in aqueous media	376	1.0–30	Ghiasvet al. (2008)
	Nitric acid in methanol media		0.5–40	
	Kinetic method	376		Mitić et al. (2007)
	p-Chloranil, in methanol medium	535	1.25×10^{-4}–2×10^{-3} $mol\ L^{-1}$	Ciapina et al. (2005)
	Concentrated nitric acid (63% w/v)	380	1.0–30	Matin et al. (2005)
	Copper (II)	680	1.0–25	De Souza and Tubino (2005)
	Iron(II)/*o*-phenanthroline	510	1.0–32	El-Didamony and Amin (2004)
	2,2-Diphenyl-1-picrylhydrazyl	–	–	Salem (2000)
	Fe^{3+}/2,2′-bipyridine	520	10–80	Agrawal and Shivramchandra (1991)
	Fe(III)/ammonium thiocyanate pH range (4.2–6.5)	481		Agatonovic-Kuštrin et al. (1997)
	p-Dimethyl-aminocinnamaldhyde	538	10–80	El Sherif et al. (1997)
	potassium ferricyanide/NaOH	450	2.0–12	Validya and Parab (1995)
	p-*N*,*N*-Dimethylphenylenediamine/$S_2O_8^{2-}$ or Cr(VI)	670	2.0–24	Sastry et al. (1989)
	Flow injection with potassium ferricyanide/sodium hydroxide	455	0.2–20	García et al. (2001)
	Flow-through sensor	281	2.0–40	Ortega-Barrales et al. (1999)
	Flow-injection with Ce(IV)–(MBTH)/H_2SO_4	580	0.20–8.0	García et al. (1998)
	Flow injection with 2,4,dichloro-6-nitrophenol (DCNP) in ethanol	450	5.0–50	Kamath et al. (1994)
	Spectrofluorimetric methods with -cyclodextrin		0.0–5.0	Arancibia et al. (2000)
	with cerium(IV) in an acidic solution		0.1243–0.600	Castillo and Bruzzone (2006)
	with Eu^{3+} ions	λ_{em} = 592 λ_{ex} = 616	10–200	Carreira et al. (1995)

Table 2 (*continued*)

Name of drug	Method	λ_{max} (nm)	Linear range ($\mu g\ mL^{-1}$)	Ref.
Etodolac	*p*-Dimethylaminobenzaldhyde reagent (PDAB)/sulfuric acid/ferric chloride	591.5	10–80	El Kousy (1999)
	Fe(III)/*o*-phenanthroline (*o*-phen)	510	0.5–8	Gouda and Hassan (2008)
	$Fe(III)^{3+}$/bipyridyl (Bipy)	520	1.0–10	
	Fe(III)/ferricyanide	725	2.0–18	
	Fe(III)/2,2′-bipyridyl	500	0.5–25	Hu et al. (2008)
	TCNE			Duymus et al. (2006)
	DDQ			
	p-CHL			
	Copper(II) acetate	684	2.0–9.0	Amer et al. (2005)
	Iron(III) chloride	385	0.5–2.0 mg mL^{-1}	
	Spectrofluorometric method	λ = 345 λ_{ex} = 235	0.096–0.640	El Kousy (1999)
Ketorolac	(MBTH)/Fe(III)	684	10–60	Shingbal and Naik (1997)
	Flow injection analysis (FIA): 2,4,dichloro-6-nitrophenol (DCNP) in methanol	450	10–120	Kamath et al. (1994)
	Spectrofluorometry in cerium(IV)/H_2SO_4	λ_{ex} = 255 λ_{em} = 365	0.1–0.8	Eid et al. (2007)

etodolac and aceclofenac. The first method is based on the formation of coloured complexes between the drugs and *p*-dimethylaminobenzaldehyde reagent (PDAB) in the presence of sulfuric acid and ferric chloride. Measurement of the absorbance was carried out at 591.5 nm for etodolac. Regression analysis of Beer's plots showed good correlation in the concentration ranges 10–80 $\mu g\ mL^{-1}$. The second was the spectrofluorometric method in which samples of etodolac in ethanol showed native fluorescence at a λ = 345 nm when excitation was at 235 nm. The calibration graph was rectilinear from 96 to 640 $ng\ mL^{-1}$. The proposed methods were applied successfully for the determination of the two drugs in bulk powder with a mean accuracy of 100.48 ± 0.85 in the PDAB method and of 99.88 ± 0.45 in the spectrofluorometric method (El Kousy, 1999).

Gouda and Hassan have described (Gouda and Hassan, 2008) three spectrophotometric methods (A–C) for the determination of etodolac in pure form and in pharmaceutical formulations. The first and second methods, A and B, are based on the oxidation of the studied drugs by Fe(III) in the presence of *o*-phenanthroline (*o*-phen) or bipyridyl (Bipy). The formation of tris-complex upon reactions with Fe(II)-*o*-phen and/or Fe(III)-Bipy mixture in an acetate buffer solution of the optimum pH-values was demonstrated at 510 and 520 nm with *o*-phen and Bipy. The third method C, is based on the reduction of iron(III) by etodolac in acid medium and subsequent interaction of iron(II) with ferricyanide to form prussian blue and the product exhibits absorption maximum at 725 nm. The concentration ranges are from 0.5 to 8.0, 1.0 to 10 and 2.0 to 18 $\mu g\ mL^{-1}$ for methods A, B and C, respectively.

A spectrophotometric method for the determination of etodolac was described. This method based on the etodolac can reduce Fe(III) to Fe(II) in the presence of 2,2′-bipyridyl (Bipy) and pH 3.5–6.0 acetate buffer medium. The Fe(II) can react with Bipy to form a Fe(II)–Bipy coloured complex. The maximum absorbance of the coloured complex is at 500 nm. Beer's law is obeyed in the range of 0.5–25 $\mu g\ mL^{-1}$ for etodolac. The method was applied to the determination of etodolac in tablets without any interference from common excipients. The RSD was 0.82% with recoveries 97–102% (Hu et al., 2008).

Spectrophotometric method for the determination of etodolac was described. This method is based on the oxidation of the studied drugs by Fe^{3+} in the presence of *o*-phenanthroline (*o*-phen) medium. The formation of tris-complex upon reactions with Fe^{3+}–*o*-phen in an acetate buffer solution of the optimum pH-values was demonstrated at 510 nm with *o*-phen. The concentration ranges are from 0.5 to 20 μg/mL for this method. The relative standard deviations were $\leqslant$0.76% with recoveries 99–101% (Ye et al., 2009).

Charge transfer (CT) complexes of etodolac, which is electron donor with some π-acceptors, such as tetracyanoethylene (TCNE), 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ), *p*-chloranil (*p*-CHL), have been investigated spectrophotometrically in chloroform at 21 °C. The coloured products are measured spectrophotometrically at different wavelength depending on the electronic transition between donors and acceptors. Beer's law is obeyed and colours were produced in non-aqueous media. All complexes were stable at least 2.0 h except for etodolac with DDQ stable for 5.0 min. The equilibrium constants of the CT complexes were determined by the Benesi–Hildebrand equation. The thermodynamic parameters ΔH, ΔS, $\Delta G°$ were calculated by Van't Hoff equation. Stoichiometry of the complexes formed between donors and acceptors were defined by the Job's method of the continuous variation and found in 1:1 complexation with donor and acceptor at the maximum absorption bands in all cases (Duymus et al., 2006).

A method depends on complexation of etodolac with copper(II) acetate and iron(III) chloride followed by extraction of complexes with dichloromethane and then measuring the extracted complexes spectrophotometrically at 684 and 385 nm in case of Cu(II) or Fe(III), respectively, was developed. Different factors affecting the reaction, such as pH, reagent concentration, and time were studied. By use of Job's method of continuous variation, the molar ratio method, and elemental analysis, the stoichiometry of the reaction was found to be in the ratio of 1:2 and 1:3, metal: drug in the case of Cu(II) and Fe(III), respectively. The method obeys Beer's

law in a concentration range of 2.0–9.0 and 0.5–2.0 mg mL^{-1} in case of Cu(II) and Fe(III), respectively. The stability of the complexes formed was also studied, and the reaction products were isolated for further investigation. The complexes have apparent molar absorptivity of about 32.14 ± 0.97 and 168.32 ± 1.12 for Cu(II) and Fe(III), respectively (Amer et al., 2005).

3.4. Ketorolac

The official method of ketorolac was potentiometric titration method with *tetrabutylammonium hydroxide* (Pharmacopoeia, 2004).

A spectrophotometric method has been developed for the estimation of ketorolac tromethamine and its dosage forms, based on its reaction with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in presence of Fe(III) ion yielding a green coloured chromogen with absorption maxima at 684 nm. Beer's law is obeyed in the concentration range of 10–60 μg mL^{-1} (Shingbal and Naik, 1997).

Ketorolac tromethamine was determined by FIA with spectrophotometric detection. The sample solution 10–120 μg mL^{-1} in methanol was injected into a flow system containing 0.01% (w/v) of 2,4,dichloro-6-nitrophenol (DCNP) in methanol. The colour produced due to the formation of a charge transfer complex was measured with a spectrophotometric detector set at 450 nm (Kamath et al., 1994).

A fluorometric method for determination of ketorolac tromethamine was studied. The method depends on oxidation of the drug with cerium(IV) and subsequent monitoring of the fluorescence of the induced cerium(III) at λ_{em} 365 nm after excitation at 255 nm. Different variables affecting the reaction conditions, such as the concentrations of cerium(IV), sulfuric acid concentration, reaction time, and temperature, were carefully studied and optimized. Under the optimum conditions, a linear relationship was found between the relative fluorescence intensity and the concentration of the investigated drug in the range of 0.1–0.8 μg mL^{-1} (Eid et al., 2007).

Table 2 shows comparison between the published spectrophotometric and spectrofluorometric methods for arylalkanoic acids.

4. Spectrophotometric and spectrofluorometric methods for determination of *N*-arylanthranilic acids derivatives (fenamic acids)

4.1. Mefenamic acid

The official method of mefenamic acid was potentiometric titration method with *sodium hydroxide* (Pharmacopoeia, 2004).

Two spectrophotometric methods for simultaneous estimation of two-component drug mixture of ethamsylate and mefenamic acid in combined tablet dosage form have been developed. The first developed method involves formation and solving of the simultaneous equation using 287.6 and 313.2 nm as two wavelengths. The second developed method is based on two wavelengths selected for estimation of mefenamic acid which were 304.8 and 320.4 nm (Goyal and Singhvi, 2008). The spectrophotometric methods for the determination of mefenamic acid and ethamsylate in pharmaceutical formulations have been developed. The methods are based on the additivity of absorbances and the determination of graphical absorbance ratio at two selected wavelengths, one being the isoabsorptive point for the two drugs (301 nm) and the other being the absorption maximum of mefenamic acid (336 nm). The Beer–Lambert's law is obeyed for mefenamic acid in the concentration range 4.0–28 μg mL^{-1} (Garg et al., 2007). Two new, simple, accurate and economical spectrophotometric methods have been developed for simultaneous estimation of drotaverine hydrochloride and mefenamic acid in two-component tablet formulation. The methods employed are, first derivative spectrophotometry, using zero crossing technique and multicomponent analysis. Both the drugs obey the Beer's law in the concentration range of 4–32 μg mL^{-1}. For quantitative estimation, absorbances were measured at λ_{max} of both the drugs viz. 279 and 308 nm for MA and DH, respectively. The assay values for tablets, were in the range of 99.15–99.30% for MA (Dahivelkar et al., 2007). A spectrophotometric method in the UV range has been developed for the simultaneous determination of mefenamic acid and paracetamol in bulk and in dosage forms. Mefenamic acid shows three absorbance maxima at 219, 284 and 336 nm in 0.1 mol L^{-1} sodium hydroxide (Dhake et al., 2001). A simultaneous spectrophotometric procedure for the determination of mefenamic acid and paracetamol in two component tablet formulations has been developed. The method is based on the two-wavelength method of calculations. The difference in absorbance at 217 and 285 nm was used for determination of mefenamic acid (Gangwal and Sharma, 1996).

In the Vierordt's spectrophotometric method, the drugs were determined by using the absorptivity values of mefenamic acid at selected wavelengths, viz., 216.8 nm, respectively. In *Q*-analysis method, isoabsorptive point was found to be at 224.6 nm. The drug obeys Beer's law in concentration range of 4–18 μg/mL (Kumar et al., 2009).

A simple visible spectrophotometric method is described for the determination of mefenamic acid in bulk sample and pharmaceutical preparations. The method is based on the reaction of mefenamic acid with *p*-*N*,*N*-dimethylphenylenediamine in the presence of $S_2O_8^{2-}$ or Cr(VI) whereby an intensely coloured product having maximum absorbance at 740 nm is developed. The reaction is sensitive enough to permit the determination of 0.25–4.0 μg mL^{-1} (Sastry et al., 1989). Spectrophotometric methods for the determination of mefenamic acid, based on the formation of a coloured species with MBTH on oxidation with Ce(IV) or Fe(III), are described (Sastry and Rao, 1989). A method for the quantitative determination of mefenamic acid in pharmaceutical preparations was proposed. The method is based on the formation of blue complexes with Folin–Ciocalteu reagent (Sastry and Rao, 1988). A spectrophotometric method was developed for the determination of mefenamic acid in the pure form and in pharmaceutical dosage forms. The method depends on their complexation with copper(II) ammonium sulphate. The complex was extracted with chloroform and treated with diethyldithiocarbamate solution, where upon another copper(II) complex (λ_{max} 430 nm) was formed. Beer's law is followed over the concentration range 6.0–48 μg mL^{-1} for mefenamic acid (Khier et al., 1987). Spectrophotometric determination of flufenamic acid, mefenamic acid, allopurinol and indomethacin using *N*-bromosuccinimide was studied (Hassib et al., 1986). Spectrophotometric determination of mefenamic acid with sodium cobaltinitrite was investigated (Sastry et al.,

1985). Mefenamic and flufenamic acids could be determined colorimetrically after extraction as ion-pairs with methylene blue (Issa et al., 1985). Extractive spectrophotometric determination of ibuprofen, ketoprofen, piroxicam, diclofenac sodium, mefenamic acid and enfenamic acid with methylene violet was illustrated (Sastry et al., 1989).

Low-cost spectrophotometric method for the determination of mefenamic acid in its pure form and pharmaceutical preparations was developed. The method is based on the charge-transfer complexation between mefenamic acid as an *n*-electron donor and chloranil as a π-acceptor to form a violet chromogen measured at 540 nm. Under the optimum conditions, a linear relationship with a good correlation coefficient (0.9996) was found between the absorbance and concentration of the studied drug in the range of 10–60 μg mL^{-1}. The LOD was 2.16 μg mL^{-1} and LOQ was 7.15 μg mL^{-1} (Raza, 2008). Mefenamic acid reacts with *p*-dimethylaminobenzaldehyde to give a bluish-green complex in acidic media after heating for 90 s at 90 °C, having maximum absorbance at 597.5 nm. The reaction is selective for mefenamic acid with 2.0 μg mL^{-1} as visual limit of quantitation and provides a basis for a new spectrophotometric determination. The reaction obeys Beer's law from 2.0 to 25 μg mL^{-1} of mefenamic acid and the RSD is 0.50% (Aman et al., 2005). A colorimetric method for the quantitative determination of mefenamic acid in pure form and in pharmaceutical preparations was developed. It was based on the interaction of the secondary aromatic amine with *p*-dimethylaminocinnamaldehyde in acidified absolute methanol medium to form very stable blue product [λ_{max} at 665 nm]. Beer's law was obeyed over the ranges 1.0–8.0 μg mL^{-1}. The reactants were heated on a boiling water bath for 5.0 min. Optimization of the different experimental conditions were studied. The mean percentage recoveries was found to be 100.73 ± 0.44%. The method was applied successfully for the determination of some pharmaceutical formulations. (El Sherif et al., 1997). Three simple, rapid and accurate spectrophotometric methods were developed for the determination of mefenamic acid. The first method (method I) is based on the reaction of mefenamic acid as *N*-donor with *p*-chloranilic acid as a π-acceptor. A red colour product shows peak at 520 nm and its absorbance is linear with concentration over the range 10–300 μg/mL with correlation coefficient (n = 12) of 0.9997. The second method (method II) involves oxidation of mefenamic acid with *N*-bromosuccinimide. A yellow colour product shows peak at 362 nm and its absorbance is linear with concentration over the range 5–70 μg mL^{-1} with correlation coefficient (n = 8) of 0.9999. The third method (method III) is based on the formation of an oxidative coupling product by the reaction of mefenamic acid with 3-methylbenzo-thiazolin-2-one hydrazone as a chromogenic reagent in presence of ferric chloride solution. A green colour product shows peak at 602 nm and its absorbance is linear with concentration over the range 1–6 μg/mL with correlation coefficient (n = 6) of 0.9999 (Alarfaj et al., 2009).

FI spectrophotometric method was proposed for the determination of mefenamic acid in bulk sample and pharmaceuticals. The method is based on the reaction of mefenamic acid with potassium ferricyanide in a sodium hydroxide medium. The absorbance of the orange product obtained is measured at 465 nm. The corresponding calibration graphs are linear over the range 1.0–100 μg mL^{-1} for mefenamic acid, while the limits of detection were 0.18 μg mL^{-1} (García et al., 2001).

A spectrofluorometric method was developed for determination of mefenamic acid in pharmaceutical preparation and human urine. The procedure is based on the oxidation of mefenamic acid with cerium(IV) to produce cerium(III), and its fluorescence was monitored at 354 nm after excitation at 255 nm. Under the experimental conditions used, the calibration graphs were linear over the range 0.03–1.5 μg mL^{-1}. The limit of detection was 0.009 μg mL^{-1} and the relative standard deviation for five replicate determinations of mefenamic acid at 1.0 μg mL^{-1} concentration level was 1.72% (Tabrizi, 2006). Terbium sensitized fluorescence was used to develop a sensitive and simple spectrofluorometric method for the determination of the anthranilic acid derivatives (mefenamic acid). The method makes use of radiative energy transfer from anthranilates to terbium ions in alkaline methanolic solutions. Optimum conditions for the formation of the anthranilate–Tb(III) complexes were investigated. Under optimized conditions, the LOD are 1.4×10^{-8} mol L^{-1}. The range of application is 2.5×10^{-8}–5.0×10^{-5} mol L^{-1}. The method was successfully applied to the determination of mefenamic acid in serum after extraction of the samples with ethyl acetate, evaporation of the organic layer under a stream of nitrogen at 40 °C and reconstitution of the residue with alkaline methanolic terbium solution prior to instrumental measurement. The mean recoveries from serum samples spiked with mefenamic acid (3.0×10^{-6}, 9.0×10^{-6} and 3.0×10^{-5} mol L^{-1}) were 101 ± 5.0 (Ioannou et al., 1998). Second-order advantage of excitation–emission fluorescence measurements was applied to the simultaneous determination of paracetamol (PC) and mefenamic acid (MF) in urine samples. Two drugs were quantified by multivariate curve resolution coupled to alternative least squares (MCR-ALS) in micellar media of sodium dodecyl sulphate (SDS). Experimental conditions including pH and SDS concentration were optimized. Under the optimum conditions, pH 2.0 and 0.05 mol L^{-1} of SDS, mefenamic acid was determined in concentration range 0.80–5.00 μg mL^{-1}, in urine samples (Madrakian et al., 2009).

4.2. Flufenamic acid

The official method of flufenamic acid was potentiometric titration method with *sodium hydroxide* (Pharmacopoeia, 2004).

A spectrophotometric method was developed for the determination of flufenamic in the pure form and in pharmaceutical dosage forms. The method depends on their complexation with copper(II) ammonium sulphate. The complex is extracted with chloroform and treated with diethyldithiocarbamate solution, whereupon another copper(II) complex (λ_{max} 430 nm) is formed. Beer's law is followed over the concentration ranges 6.0–60 μg mL^{-1} for flufenamic acid (Khier et al., 1987).

Spectrofluorometric method for determination of flufenamic acid in bulk powder and capsule dosage forms was presented. The methods are based on the cyclization reaction of flufenamic acid with concentrated sulfuric acid to produce the corresponding acridone derivative and measurement of the fluorescence intensity at 450 nm (λ_{ex} = 400 nm) and peak-to-peak measurements of the first- (^{1}D) and second-derivative (^{2}D) curves, respectively. Beer's law is obeyed over the concentration ranges of 2.0–20 ng mL^{-1} (Sabry and Mahgoub, 1999).

4.3. Enfenamic acid

The official method of enfenamic acid was potentiometric titration method with *sodium hydroxide* (Pharmacopoeia, 2004).

Spectrophotometric method for the quantitative determination of enfenamic acid and naproxen (after demethylation) based on the formation of a coloured oxidative coupling product with 2,6-dichloro-*p*-benzoquinone-4-chlorimine (Gibb's reagent) in phosphate buffer (pH 7.0) was developed. The reaction is sensitive to permit the determination of 0.25 μg mL^{-1} of enfenamic (Sastry et al., 1988).

A simple visible spectrophotometric method was described for the determination of enfenamic acid in bulk samples and pharmaceutical preparations. The method is based on the reaction of enfenamic acid with *p*-*N*,*N*-dimethylphenylenediamine in the presence of $S_2O_8^{2-}$ or Cr(VI) whereby an intensely coloured product having maximum absorbance at 720 nm is developed. The reaction is sensitive enough to permit the determination of 0.125–2.0 μg mL^{-1} (Sastry et al., 1989).

4.4. Tolfenamic acid

The official method of tolfenamic acid was potentiometric titration method with *sodium hydroxide* (Pharmacopoeia, 2004).

Terbium sensitized fluorescence was used to develop a sensitive and simple spectrofluorimetric method for the determination of the anthranilic acid derivative (tolfenamic acid). The method makes use of radiative energy transfer from anthranilates to terbium ions in alkaline methanolic solutions. Optimum conditions for the formation of the anthranilate–Tb(III) complexes were investigated. Under optimized conditions, the LOD was 9.0×10^{-9} mol L^{-1}. The range of application is 2.5×10^{-8}–5.0×10^{-5} mol L^{-1}. The method was successfully applied to the determination of tolfenamic acid in serum after extraction of the samples with ethyl acetate, evaporation of the organic layer under a stream of nitrogen at 40 °C and reconstitution of the residue with alkaline methanolic terbium solution prior to instrumental measurement. The mean recoveries from serum samples spiked with tolfenamic

Table 3 Comparison between the spectrophotometric methods for determination of *N*-arylanthranilic acids (fenamic acids).

Name of drug	Method	λ_{max} (nm)	Linear range (μg mL^{-1})	Ref.
Mefenamic acid	UV methods	336 nm	4.0–28	Garg et al. (2007)
		219, 284 and 336		Dhake et al. (2001)
		217 and 285		Gangwal and Sharma (1996)
	p-*N*,*N*-Dimethylphenylenediamine/$S_2O_8^{2-}$ or Cr(VI)	740	0.25–4.0	Sastry et al. (1989)
	MBTH/Ce(IV) or Fe(III)			Sastry and Rao (1989)
	Folin–Ciocalteu			Sastry and Rao (1988)
	Copper(II) ammine sulphate/ diethyldithiocarbamate	430	6.0–48	Khier et al. (1987)
	N-Bromosuccinimide			Hassib et al. (1986)
	Sodium cobaltinitrite			Sastry et al. (1985)
	Methylene blue			Issa et al. (1985)
	Methylene violet			Sastry et al. (1989)
	Chloranil	540	10–60	Raza (2008)
	p-Dimethylaminobenzaldehyde	597.5	2.0–25	Aman et al. (2005)
	p-Dimethylaminocinnamaldehyde	665	1.0–8.0	El Sherif et al. (1997)
	p-Chloranilic acid	520	10–300	Alarfaj et al. (2009)
	N-Bromosuccinimide	362	5–70	
	3-Methylbenzo-thiazolin-2-one hydrazone as + ferric chloride	602	1–6	
	Flow injection with potassium ferricyanide/ sodium hydroxide	465	1.0–100	García et al. (2001)
	Spectrofluorometric methods with cerium(IV) in an acidic solution	λ_{em} = 354	0.03–1.5	Tabrizi (2006)
		λ_{ex} = 255		
	with terbium Tb(III)		2.5×10^{-8}–5.0×10^{-5} mol L^{-1}	Ioannou et al. (1998)
Flufenamic acid	Copper(II) ammine sulphate/ diethyldithiocarbamate	430	6.0–60	Sabry and Mahgoub (1999)
	Spectrofluorometry with concentrated sulfuric acid	λ_{em} = 450	2.0–20 ng mL^{-1}	Khier et al. (1987)
		λ_{ex} = 400		
Enfenamic acid	2,6-Dichloro-*p*-benzoquinone-4-chlorimine			Sastry et al. (1988)
	p-*N*,*N*-Dimethylphenylenediamine/$S_2O_8^{2-}$	720	0.125–2.0	Sastry et al. (1989)
Tolfenamic acid	Spectrofluorometry with terbium Tb(III)		2.5×10^{-8}–5.0×10^{-5} mol L^{-1}	Ioannou et al. (1998)

acid (3.1×10^{-6}, 12.5×10^{-6} and 2.5×10^{-5} mol L^{-1}) were 98 ± 7.0% (Ioannou et al., 1998).

Table 3 shows comparison between the published spectrophotometric and spectrofluorometric methods for fenamic acids.

5. Spectrophotometric and spectrofluorometric methods for determination of arylpropionic acids (profens)

5.1. Ibuprofen

The official method of ibuprofen was potentiometric titration method with *sodium hydroxide* (Pharmacopoeia, 2004).

The simultaneous determination of paracetamol, ibuprofen and caffeine in pharmaceuticals by chemometric approaches using UV spectrophotometry has been reported as a simple alternative to using separate models for each component. Spectra of paracetamol, ibuprofen and caffeine were recorded at several concentrations within their linear ranges and were used to compute the calibration mixture between wavelengths 200 and 400 nm at an interval of 1.0 nm in methanol: 0.1 mol L^{-1} HCl (3:1). Partial least squares regression (PLS), genetic algorithm coupled with PLS (GA-PLS), and principal component-artificial neural network were used for chemometric analysis of data and the parameters of the chemometric procedures were optimized. The analytical performances of these chemometric methods were characterized by relative prediction errors and recoveries (%) and were compared with each other (Khoshayand et al., 2008). A spectrophotometric method for the simultaneous and separate estimation of ibuprofen and paracetamol in binary tablet formulation has been developed. This method is based on the estimation of one drug in presence of another drug by absorbance difference method. The ibuprofen and paracetamol solution were scanned over a range of 200–600 nm. In this method, two wavelengths 220 and 231 nm were chosen for ibuprofen and at these wavelengths the absorbance difference was almost zero while there was considerable absorbance difference in case of paracetamol, similarly. The amount of ibuprofen was directly proportional to the absorbance difference between 241 and 255 mm (Omry et al., 2007). A simple method was proposed for determination of paracetamol and ibuprofen in tablets, based on UV measurements and partial least squares. The procedure was performed at pH 10.5, in the concentration range 2.4–12.0 μg mL^{-1} (ibuprofen). The model was able to predict paracetamol and ibuprofen in synthetic mixtures with root mean squares errors of prediction of 0.17 μg mL^{-1} (Sena et al., 2007). Spectrophotometric methods were described for the simultaneous determination of pseudoephedrine hydrochloride and ibuprofen in their combination. The obtained data were evaluated by using five different methods. In the first method, ratio spectra derivative spectrophotometry, analytical signals were measured at the wavelengths corresponding to either maximums and minimums for both drugs in the first derivative spectra of the ratio spectra obtained by using each other spectra as divisor in their solution in 0.1 mol L^{-1} HCl. In the other four methods using chemometric techniques, classical least-squares, inverse least-squares, principal component regression and partial least-squares (PLS), the concentration data matrix were prepared by using the synthetic mixtures containing these drugs in methanol: 0.1 mol L^{-1} HCl (3:1). The absorbance data matrix corresponding to the concentration data matrix was obtained by the measurements of absorbance in the range 240–285 nm in the intervals with $\Delta\lambda = 2.5$ nm at 18 wavelengths in their zero-order spectra, then, calibration or regression was obtained by using the absorbance data matrix and concentration data matrix for the prediction of the unknown concentrations of pseudoephedrine hydrochloride and ibuprofen in their mixture. The procedures did not require any separation step. The linear range was found to be 300–1300 μg mL^{-1} in all five methods (Palabiyik et al., 2004). Two procedures for simultaneous estimation of ibuprofen and methocarbamol in two component tablet formulation have been developed. Solutions were prepared in 0.1 mol L^{-1} sodium hydroxide using all glass double distilled water. Ibuprofen has an absorbance maximum at 222 nm (Manikandan et al., 2001). The second-derivative spectrophotometric method for the simultaneous determination of pseudoephedrine in the combinations with ibuprofen was described. The second-derivative order of the spectra in ethanol with the wavelength modulation was used. For the quantitative assay for all of the investigated substances in the laboratory mixture or in respective pharmaceutical dosage forms, the 'zero-crossing' technique was applied (Ivanovic et al., 2000). A spectrophotometric method requiring no prior separation has been developed. The method employs first derivative ultraviolet spectrophotometry for the simultaneous estimation of ibuprofen and dextropropoxyphene hydrochloride. In aqueous methanol (10% v/v), ibuprofen has a maximum at 256 nm. In derivative spectroscopy, estimation of ibuprofen was carded out in first order with $N = 6$ at 232 nm (Sachan and Trivedi, 1998). Reproducible method for estimation of ibuprofen and pseudoephedrine hydrochloride in combined dosage form has been developed. The method involves two-wavelength calculation. The two wavelengths selected for estimation of ibuprofen are 264.0 and 254.5 nm (Singhvi and Chaturvedi, 1998a). Two methods for simultaneous estimation of ibuprofen and pseudoephedrine hydrochloride in combined dosage form have been developed. First developed method employs formation and solving of simultaneous equations using 263.8 and 257.6 nm as two wavelengths for formation of equations. Second method involves first derivative ultraviolet spectroscopy. Two wavelengths selected for this method are 265 and 257 nm (Singhvi and Chaturvedi, 1998b).

A new extractive spectrophotometric method for the determination of ibuprofen was developed. The method involves the formation of coloured electron donor–acceptor complex between ibuprofen and safranine in the aqueous phase extractable into chloroform, which is measured at λ_{max} 520 nm. This method is extended to pharmaceutical dosage forms (Babu, 1998). Kinetic spectrophotometric method for the determination of ibuprofen in pharmaceutical formulations. Ibuprofen was determined in an acidic ethanolic medium by monitoring the rate of appearance of 1-nitroso-2-naphthol, resulting from the displacement by ibuprofen of Co(III) from the tris(1-nitroso-2-naptholato)cobalt(III) complex. The optimum operating conditions regarding reagent concentrations and temperature were established. The tangent method was adopted for constructing the calibration curve, which was found to be linear over the concentration range 0.21–1.44 and 1.44–2.06 μg mL^{-1} (Mitić et al., 2008).

A spectrophotometric method was presented for the determination of ibuprofen by batch and flow injection analysis methods. The method is based on ibuprofen competitive

complexation reaction with phenolphthalein-β-cyclodextrin (PHP-β-CD) inclusion complex. The increase in the absorbance of the solution at 554 nm by the addition of ibuprofen was measured. Ibuprofen can be determined in the range 8.0×10^{-6}–3.2×10^{-4} and 2.0×10^{-5}–5.0×10^{-3} mol L^{-1} by batch and flow methods, respectively. The LOD and LOQ were 6.19×10^{-6} and 2.06×10^{-5} mol L^{-1} for batch and 1.77×10^{-5} and 5.92×10^{-5} mol L^{-1} for flow method, respectively. The sampling rate in flow injection analysis method was 120 ± 5.0 samples h^{-1}. The method was applied to the determination of pharmaceutical formulations (Afkhami et al., 2007).

The inclusion complexation of ibuprofen with β-CD has been examined by means of spectrofluorometry at both acid and alkaline pH. The results suggest that stable 1:1 complexes are formed in both media. The analysis of the pK_a values for ibuprofen in both the absence and presence of β-CD (4.12 and 4.66, respectively) suggests that in the inclusion complex the carboxylic group is located outside the α-cyclodextrin (α-CD) but interacting with it. Further structural characterization of the complex was carried out by means of AM1 semiempiral calculations. Based on the obtained results, a spectrofluorometric method for the determination of ibuprofen in the presence of β-CD at 10 °C was developed in the range of 4.7–58 μg mL^{-1}. Better LOD (1.6 μg mL^{-1}) and LOQ (4.7 μg mL^{-1}) were obtained in this latter case with respect to those obtained in the absence of β-CD. The method was satisfactorily applied to the quantification of ibuprofen in pharmaceutical preparations. A novel spectrofluorometric determination of ibuprofen in the presence of β-CD was also developed for serum samples at concentration levels between 5.0 and 70 μg mL^{-1} (Hergert and Escandar, 2003).

The characteristics of host–guest complexation between β-cyclodextrin (β-CD) and two forms of ibuprofen (protonated and deprotonated) were investigated by fluorescence spectrometry. Stoichiometry for both complexes were established to be 1:1 and their association constants at different temperatures were calculated by applying a non-linear regression method to the change in the fluorescence of ibuprofen that was brought about by the presence of β-CD. The thermodynamic parameters (ΔH, ΔS and $\Delta G°$) associated with the inclusion process were also determined. Based on the obtained results, a sensitive spectrofluorometric method for the determination of ibuprofen was developed with a linear range of 0.1–2.0 μg mL^{-1} with LOD of 0.03 μg mL^{-1} (Manzoori and Amjadi, 2003).

The spectrofluorometric determination of ibuprofen in pharmaceutical tablets, creams and syrup is described. It involves excitation at 263 nm and emission at 288 nm. The linear range is 2.0–73 μg mL^{-1} (Damiani et al., 2001).

Luminescence properties of the complexes of terbium(III) with ibuprofen and orthofen were studied. It was demonstrated that in the presence of organic bases (2,2′-dipyridyl and *o*-phenanthroline) mixed-ligand complexes are formed and the luminescence intensity of terbium(III) increases by a factor of up to 250. The LOD are 2.0 and 0.05 μg mL^{-1}, respectively (Teslyuk et al., 2007).

5.2. Ketoprofen

The official method of ketoprofen was potentiometric titration method with *sodium hydroxide* (Pharmacopoeia, 2004).

A binary mixture of hyoscine butylbromide and ketoprofen was determined by four different methods. The first involved determination of ketoprofen was by using the ratio-spectra first-derivative spectrophotometric technique at 234 nm over the concentration ranges of 5.0–45 μg mL^{-1}. The second method utilized second-derivative spectrophotometry over the concentration ranges of 5.0–35 μg mL^{-1} with mean accuracies 99.55 ± 1.15%, respectively. The third method was based on the resolution of the two components by bivariate calibration depending on a simple and rapid mathematical algorithm and quantitative evaluation of the absorbance at 254 nm over concentration ranges of 5.0–35 μg mL^{-1}; mean accuracies of 100.19 ± 1.07% were obtained for ketoprofen. The fourth method was reversed-phase liquid chromatography using 0.05 mol L^{-1} ammonium dihydrogen phosphate–acetonitrile–methanol (20 + 30 + 6, v/v) as the mobile phase with ultraviolet detection at 220 nm over concentration ranges of 1.0–90 and 5.0–70 μg mL^{-1}; mean accuracies were 99.92 ± 1.02% and 99.61 ± 0.98%, for hyoscine butylbromide and ketoprofen, respectively (El-Saharty et al., 2007). Partial least-squares calibration was used for the simultaneous UV spectrophotometric determination of the active principle (ketoprofen) and preservative (parabens) in a pharmaceutical preparation commercially available in gel form. Calibration mixtures were prepared by mixing pure solutions of the analytes (Blanco et al., 1997). A second order derivative spectrophotometric method was developed for the permeative determination of ketoprofen in vitro. The method can avoid the disturbance of skin tissue (Hu et al., 1997). The mean recovery of ketoprofen is 99.00 ± 1.51%.

A spectrophotometric determination of ketoprofen based upon oxime formation followed by charge transfer complexation with *o*-chloranil has been developed. Different variables affecting the complexation process have been studied. Beer's law is obeyed in the concentration range 10–80 μg mL^{-1} (El-Sadek et al., 1993).

5.3. Flurbiprofen

The official method of flurbiprofen was potentiometric titration method with *sodium hydroxide* (Pharmacopoeia, 2004).

A UV spectrophotometric method for quantitative estimation of flurbiprofen in pure form and in pharmaceutical dosage forms was developed. The linear regression equations obtained by least square regression method were Abs = 7.5906×10^{-2} concentration (μg mL^{-1}) ± 4.6210×10^{-2} for the UV method. The detection limit as per the error propagation theory was found to be 0.34 μg mL^{-1} for UV method (Sajeev et al., 2002).

5.4. Naproxen

The official method of naproxen was potentiometric titration method with *sodium hydroxide* (Pharmacopoeia, 2004).

A second-derivative spectrophotometric method for the determination of naproxen in the absence or presence of its 6-desmethyl metabolite in human plasma is described. The method consists of direct extraction of the non-ionized form of the drug with pure diethyl ether and determination of the naproxen by measuring the peak amplitude (mm) in the second-order derivative spectrum at a wavelength of 328.2 nm. The efficiency of the extraction procedure expressed by the absolute recovery was 94.6 ± 0.7% (mean ± SD) for the concentration range tested, and the LOQ attained according to the IUPAC definition was 2.42 μg mL^{-1} (Panderi and Parissi-Poulou, 1994).

Table 4 Comparison between the spectrophotometric methods for determination of arylpropionic acids (profens).

Name of drug	Method	λ_{max} (nm)	Linear range ($\mu g\ mL^{-1}$)	Ref.
Ibuprofen	UV methods	200 and 400		Khoshay et al. (2008)
		220 and 231		Omry et al. (2007)
			2.4–12.0	Sena et al. (2007)
		240–285	300–1300	Palabiyik et al. (2004)
		222		Manikandan et al. (2001)
		256		Sachan and Trivedi (1998)
		264 and 254.5		Singhvi and Chaturvedi (1998a)
		263.8 and 257.6		Singhvi and Chaturvedi (1998b)
	Safranine	520		Babu (1998)
	Kinetic method		0.21–2.06	Mitić et al. (2008)
Ketoprofen	First-derivative	234	5.0–45	El-Saharty et al. (2007)
	Second-derivative		5.0–35	
	o-Chloranil		10–80	El-Sadek et al. (1993)
Naproxen	Second-derivative	328.2		Panderi and Parissi-Poulou (1994)
	1-Naphthylarnine and sodium nitrite	460–480	10–65	Khan et al. (1999)
	MBTH with Ce(VI) or Fe(III)			Hassan et al. (2008)
	2,6-Dichloro-*p*-benzoquinone-4-chlorimine (gibbs reagent)			Jaiswal et al. (2007)
	TCNE, DDQ, *p*-CHL			Duymus et al. (2006)
Tiaprofenic Acid	Safranine-T			Vinci et al. (2006)

Naproxen reacts with 1-naphthylarnine and sodium nitrite to give an orangish red colour having maximum absorbance at 460–480 nm (working wavelength 480 nm). The reaction is selective for naproxen with 0.001 mg mL^{-1} as visual LOQ and provides a basis for a new spectrophotometric determination. The reaction obeys Beer's law from 10 to 65 μg mL^{-1} of naproxen and the relative standard deviation is 1.5%. The quantitative assessment of tolerable amount of other drugs is also studied (Khan et al., 1999). Spectrophotometric methods for the determination of naproxen based on the formation of a coloured species with MBTH on oxidation with Ce(IV) or Fe(III), are described (Sastry and Rao, 1989). Spectrophotometric method for the quantitative determination of naproxen, after demethylation was developed based on the formation of a coloured oxidative coupling product with 2,6-dichloro-*p*-benzoquinone-4-chlorimine (Gibb's reagent) in phosphate buffer (pH 7.0). The reaction is sensitive to permit the determination of 5.0 μg mL^{-1} (Sastry et al., 1988).

CT complexes of naproxen, which is electron donor with some π-acceptors, such as tetracyanoethylene (TCNE), 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ), *p*-chloranil, have been investigated spectrophotometrically in chloroform at 21 °C. The coloured products are measured spectrophotometrically at different wavelength depending on the electronic transition between donors and acceptors. Beer's law is obeyed and colours were produced in non-aqueous media. All complexes were stable at least 2.0 h except for etodolac with DDQ stable for 5.0 min. The equilibrium constants of the CT complexes were determined by the Benesi–Hildebrand equation. The thermodynamic parameters ΔH, ΔS, $\Delta G°$ were calculated by Van't Hoff equation (Duymus et al., 2006).

5.5. Tiaprofenic acid

The official method of tiaprofenic acid was potentiometric titration method with *sodium hydroxide* (Pharmacopoeia, 2004).

The spectrophotometric method for the determination of tiaprofenic acid was described. The method depends on the determination of the drug after extraction as an ion–association complex with safranine-T in chloroform at pH 7.4 (Ali et al., 1994).

Table 4 shows comparison between the published spectrophotometric and spectrofluorometric methods for profens.

6. Applications

The above mentioned methods have applications in the determination of the studied drugs in various pharmaceutical formulations like tablets, suppositories, injections, capsules and oral solutions. These methods give results which are comparable with the official pharmacopoeial methods used for the determination of the studied non-steroidal anti-inflammatory; hence, these methods can be successfully used for routine analysis and quality control of non-steroidal anti-inflammatory drugs. The methods have been used for the quantitative determination of the drug in pure form and commercial preparations. Human urine samples and serum samples have also been successfully analyzed for the studied non-steroidal anti-inflammatory drugs by these methods. The commonly occurring exicipients do not interfere in the determination of the drug in the case of commercial samples. The methods have been validated statistically compared with the official methods by applying student's *t*-test and *F*-value. The results have been found to be accurate, precise and comparable to the official methods.

7. Conclusions

This review presents spectrophotometric and spectrofluorometric analytical methods applied for the determination of some non-steroidal anti-inflammatory drugs, (coxibs, arylalkanoic acids, *N*-arylanthranilic acids (fenamic acids) and 2-arylpropionic acids (profens)) between 1985 and 2008.

Spectrophotometric methods in UV–vis (classical and for consecutive derivatives) as well as fluorometric are also quite common, being most frequently used for quantification or confirmation of substance identity. Despite wide availability of the equipment, their use is however still limited, especially with a complicated matrix. The ultimate goal is to obtain results with more and more precision and accuracy and at increasingly lower concentration levels of the substances being determined. This also facilitates the course of analysis by reducing the impact of the matrix, without prior labour-consuming preparation of the samples (especially the biological ones). Comparing validation parameters of already researched methods, it can be concluded which one of them is more sensitive (low LOD and LOQ values), accurate (precision and recovery) and allows markings in a broad linearity scope.

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