REVIEW

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Determination of non-steroidal anti-inflammatory drugs in biological fluids

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The present paper summarizes the methods of determination of non-steroidal anti-inflammatory drugs in biological fluids. CZE, HPLC, HPTLC, GC-MS are analytical techniques, which are capable of a separation of arylpropionic acids.

1. Introduction

The arylpropionic acids (APAs) **1**–**19** represent an important group of non-steroidal anti-inflammatory drugs (Table). All these compounds are chiral. Their activity resides mainly in the enantiomers with the *S*-configuration. The difference in activity for most APAs is compensated by metabolic inversion of the (*R*)-(−)-to the (*S*)-(+)-enantiomer [1, 2]. The analysis of the different enantiomeric forms of chiral molecules is an area of increasing importance in separation science. In the more conventional chromatographic procedures such as HPLC, GC, and TLC, chiral separation is achieved by the use of chiral additives in the mobile phase or by a chiral stationary phase (CSP). The separation and the quantification of individual enantiomers in biological material requires high selectivity, firstly to discriminate the analytes from matrix components, and secondly to separate the optical isomers. This is especially pronounced at low concentrations of the analyte. In the field of capillary electrophoresis (CE), chiral separations are being undertaken by the use of chiral additives in the running buffer. Davies reviewed the mtehods of analysis of chiral non-steroidal anti-inflammatory drugs [3].

Many techniques have been used for the analysis of drug standards or pharmaceuticals such as enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), supercritical fluid chromatography (SFC), GC-MS, HPLC and optical methods.

There are many of publications reporting on the determination of NSAID substances in biological fluids, however, up to the present time, a review of NSAID determinations in biological fluids has not appeared. The concentration for therapeutic activity during steady state are as reported: for fenoprofen 15–65 mg/l, for ibuprofen 15–30 mg/l, for ketoprofen 1–5 mg/l, for naproxen 25–75 mg/l, for tiaprofenic acid 15–35 mg/l [73].

2. High performance liquid chromatography

2.1. Urine

A sequential achiral-chiral HPLC method has been developed for the stereospecific analysis of the two major urinary metabolites of ibuprofen, namely hydroxyibuprofen and carboxyibuprofen. Achiral analysis was carried out a Partisil column and mobile phase of hexane-ethanol (98.2 : 1.8, v/v) containing trifluoroacetic acid (0.05%, v/v). Chiral-phase analysis was carried out using a Chiralpak AD CSP with a mobile phase pf hexane-ethanol (92 : 8) containing trifluoroacetic acid (0.05%, v/v). Two males and one female were administered a single oral dose of racemic ibuprofen as a 400 mg tablet, following an overnight fast. The enantiomeric composition of ibuprofen in urine both free and conjugated with glucuronide acid was determined. Following extraction, the drug was derivatized with (*R*)-1-(naphten-1-yl)-ethylamine and the enantiomeric composition by RP-HPLC. The limit of quantitation for procedure was 10 μg/ml for both metabolites in urine [4].

Table: Arylpropionic acids

Compd.	Generic name	Systematic name
1	Alminoprofen	(±)-(2-[p-(Methallylamino)phenyl)propionic acid
2	Benoxaprofen	(±)-2-[2-(p-Chlorophenyl)-5-benzoxazolyl]propionic acid
3	Bermoprofen	(±)-10,11-Dihydro-α,8-dimethyl-11-oxo-dibenz[b,f]oxepin-2-acetic acid
4	Carprofen	(±)-2-(6-Chloro-2-carbazolyl)propionic acid
5	Cicloprofen	(±)-2-(2-Fluorenyl)propionic acid
6	Fenoprofen	(±)-2-(3-Phenoxyphenyl)propionic acid
7	Flunoxaprofen	(±)-2-[2-(p-Fluorophenyl)-5-benzoxazolyl]propionic acid
8	Flurbiprofen	(±)-2-(2-Fluoro-4-biphenyl)propionic acid
9	Ibuprofen	(±)-2-(p-Isobutylphenyl)propionic acid
10	Indoprofen	(±)-2-[4-H-Oxo-2-isoindolinyl)phenyl]propionic acid
11	Ketoprofen	(±)-2-(3-Benzoylphenyl)propionic acid
12	Loxoprofen	(±)-2-[4-[(2-Oxocyclopentyl)methyl)phenyl]propionic acid
13	Miroprofen	(±)-2(p-Imidazo[1,2-a]pyridin-2-ylphenyl)propionic acid
14	Naproxen	(±)-2-(6-Methoxy-2-naphthyl)propionic acid
15	Pirprofen	(±)-2-[3-Chloro-4-(3-pyrrolin)-1-yl)phenyl]propionic acid
16	Pranoprofen	(±)-2-(5 H-[1]Benzopyrano[2,3-b]-pyridin-7-yl]propionic acid
17	Suprofen	(±)-2-[4-(2-Thenoyl)-phenyl]propionic acid
18	Tiaprofenic acid	(±)-2-(5-Benzoyl-2-thienyl)propionic acid
19	Ximoprofen	(±)-2-[4-[3-(Hydroxyimino)cyclohexyl]phenyl]propionic acid

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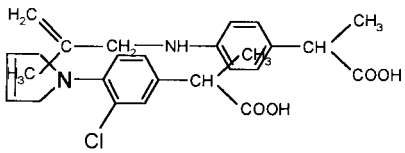

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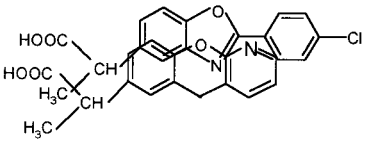

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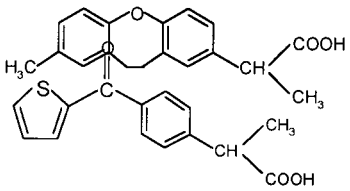

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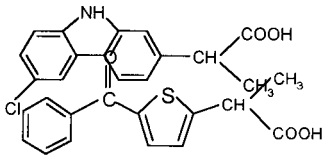

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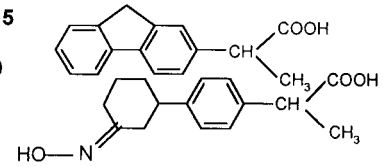

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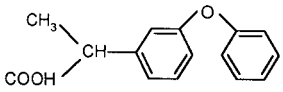

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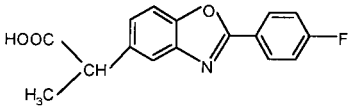

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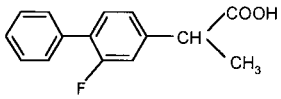

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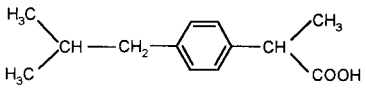

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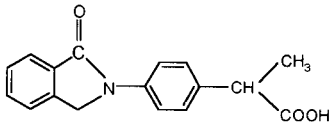

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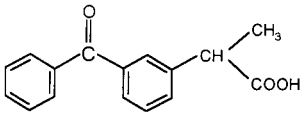

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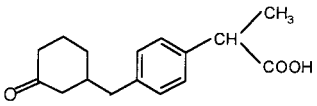

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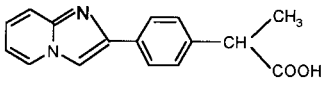

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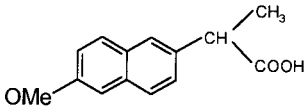

Keep et al. reported an HPLC method for the simultaneous determination of the major metabolites of ibuprofen as well as the glucuronic acid conjugates of ibuprofen and its major phase I metabolites in human urine. The separation was performed using bare silica dynamically modified with *N*-cetyl-*N*,*N*,*N*-trimethylammonium hydroxide ions contained in the mobile phase. Four healthy volunteers were each given 600 mg of ibuprofen orally. The detection limits for β-1-O-acyl glucuronic acid conjugat of 2-hydroxyibuprofen (Ibu-2-OH-gclU), 2-hydroxyibuprofen (Ibu-2-OH), β-1-O-acyl glucuronic acid conjugat of carboxyibuprofen (Ibu-COOH-gclU) were 1 μg/ml, for β-1-O-acyl glucuronic acid conjugat of ibuprofen (Ibu-gclU) was 12 μg/ml and Carboxyibuprofen (Ibu-COOH) was 5 μg/ml in urine. The limits of quantitation were for Ibu-2-OH-gclU and Ibu-2-OH 4 μg/ml, for Ibu-COOH-gclU was 5 μg/ml, Ibu-COOH was 17 μg/ml and Ibu-gclU 23.2 μg/ml. A total recovery in urine of the dose given was only 58–86% within 8 h [5].

2.2. Serum and plasma

A simple, sensitive and reliable HPLC method for the determination of naproxen in dog serum after administration of a topical gel is presented by Shu et al. Samples were prepared by a protein precipitation method using acetonitrile. The limit of quantitation was 2.0 ng/ml [6]. Multisite binding of fenoprofen to human serum-albumin was studied by a combined technique of microdialysis with HPLC [7].

To investigate the glucuronidation of the *R*- and *S*-enantiomers of flurbiprofen by liver microsomes of several mammals, including humans, a new and reliable HPLC method for the separation of the diastereomeric glucuronides has been developed [8]. Chi et al. reported the analysis of flurbioprofen in rat plasma [9]. Determination of flurbiprofen in rat plasma using HPLC with fluorescence detection was presented by Park et al. The detection limit in rat plasma was 50 ng/ml. The mean percentage recovery of the drug was 95.1% [10]. Stereoselective separation achieved with a prepacked α_1-acid glycoprotein column without any derivatization procedure was reported. The utility of the method was demonstrated after oral administration of 50 mg of *R*- and *S*-flurbiprofen to healthy volunteers. The quantification limit in human plasma was found to be 50 ng/ml for the stereoselective and the racemic method [11].

Determination of ibuprofen enantiomers in human plasma by precolumn derivatization and HPLC with fluorescence detection has been demonstrated. The method is based on the separation of the diasteromers formed on reacting ibuprofen enantiomers and fenoprofen (internal standard) with *S*-(−)-1-(1-naphthyl)ethylamine [12]. A derivatization HPLC method with a chiral reagent (−)-2-[4-(-aminoethyl)phenyl]-6-methoxybenzoxazole in the presence of 2,2-dipyridyl disulphide and triphenylphosphine in dichloromethane was described. The minimum quantifiable concentrations in rat plasma were 0.2 μg/ml for *S*-ibuprofen and 0.4 μg/ml for *R*-ibuprofen, respectively, in a 10 μl volume. Liquid-liquid (L-L) extraction is still a popular sample preparation technique, however L-L extraction is not adequate when dealing with microvolume samples [13].

Ibuprofen was determined in erythrocytes and plasma of rabbits by HPLC after isolation by liquid-liquid extraction from these biological matrices. Methylene chloride proved to be the best of the organic solvents tested. This method has been applied to a pharmacokinetic study [14]. Another investigator also reports the determination of ibuproxam and its metabolite ibuprofen in human plasma. The detection limit was 0.1 μg/ml [15]. A reversed-phase HPLC method for the direct, simple, simultaneous determination of ibuprofen and its labile, reactive metabolite, ibuprofen acyl glucuronide, in human plasma is described. The validation parameters showed a quantitative recovery, minimal chromatographic baxckground, good intra- and inter-day reproducibility, linearity over 0.5–100 μg/ml for ibuprofen. The application of the method was demonstrated after oral administration of 800 mg of ibuprofen to one human volunteer. The limit of detection was 0.1 μg/ml [16].

Sochor et al. used reverse-phase HPLC with UV detection to analyse ibuprofen in whole-blood using solid-phase extraction. Ibuprofen was isolated on C_{18} extraction columns. The method was used in pharmacokinetic studies in rabbits. Of the eluents tested, methylene chloride was preferred to methanol because the latter gave a low extraction efficiency. Generally, limits of detection are lower than with simple precipitation sample preparations, and for many compounds limits of detections around 0.1 μg/ml can be achieved even with UV detection. In order to increase the efficiency of the solid phase extraction (SPE), it was necessary to carry out hemolysis of the blood sample. The limit of detection for ibuprofen in blood was 100 ng/ml and the limit of quantitation was 300 ng/ml. Rabbits were treated with a solution of ibuprofen (25 mg/kg weight). The advantage of this method is that whole-blood samples can be analyzed. After withdrawing the blood sample, it is not necessary to separate the plasma; the samples can be directly analyzed [17].

Ibuprofen was tagged with 4-(*N*,*N*-dimethylaminosulphonyl)-7-*N*-piperazino-2,1,3-benzoxadiazole in the presence of diethylphosphorocyanidate and separated by isocratic elution. The limit of detection was 4.3 fmol [18]. Measurement of ibuprofen in plasma has been accomplished with an HPLC apparatus [19]. High doses of ibuprofen have been shown to delay the progression of lung disease without serious adverse effects in patients with cystic fibrosis. Rafai et al. developed an HPLC assay to rapidly determine plasma ibuprofen concentrations. The assay proved to be free of interference from 51 medications [20]. Concentrations of *R*-ibuprofen, *S*-ibuprofen and its metabolites were monitored in rat plasma and lymph by HPLC by Oelkers et al. [21].

A HPLC system for the stereoselective determination of unbound ketoprofen enantiomers in plasma was developed. After direct sample injection a low level of unbound ketoprofen that was in equilibrium with ketoprofen bound to protein could be enantioselectively determined. The advantage of this method over the widely used ultrafiltration method is that at a very low concentration of unbound drug, due to strong binding to protein, can be determined enantioselectively without sample pretreatment. Undesirable adsorption of drug and leakage of proteins, which often cause problems in an ultrafiltration method, can be avoided. The limit of detection was found to be 1.0 nM for each ketoprofen enantiomer [22]. The HPLC assay involves an extraction of the ketoprofen from acidified human plasma which is then back-extracted into a small volume of alkaline aqueous solution before injection onto HPLC column. The highly sensitive nature of this method was applied successfully to the determination of ketoprofen in human plasma for pharmacokinetic studies [23].

The use of HPLC for the determination of ketoprofen in plasma was reported [24, 25]. Online determination and resolution of enantiomers of ketoprofen in plasma through

use of coupled achiral chiral HPLC was shown. This method is greatly superior to usual column-switching HPLC [26]. The method described is capable of separating ketoprofen enantiomers in human plasma with direct resolution of enantiomers using a commercially available chiral stationary phase that offers a simple, sensitive and fast alternative for ketoprofen determination. This method was applied to a pharmacokinetic study on healthy human volunteers following the administration of a ketoprofen extended-release product (200 mg of ketoprofen tablet) [27].

A simple, direct procedure is described for the analysis of *R*- and *S*-ketoprofen and their glucuronides, following alkaline hydrolysis, which is sufficiently sensitive and robust to be applied to the plasma of patients with chronic renal insufficiency. The system I used employed a mobile phase of acetonitrile-potassium phosphate buffer, 50 mM, pH = 3.0, containing the ion-pair reagent tetrabutyl ammonium hydrogen sulphate at UV absorbance detection at 254 nm. In the absence of the ion-pair reagent, *R*- and *S*-ketoprofen glucuronides eluted as a single peak. System I was used for the analysis and preparative isolation of *R*- and *S*-ketoprofen glucuronides in human urine, which were then use as standards. System IV was used for the analysis of conjugated *R*- and *S*-ketoprofen, *R*- and *S*-ketoprofen after ketoprofen glucuronide hydrolysis. They were separated using a S,S-Whelk-0 1, 5 μm particle size column and detected by UV absorbance at 254 nm. The mobile phase consisted of hexane-ethanol (90 : 10 v/v) containing 0.1% acetic acid. The limit of determination was 20 ng/ml in plasma for each enantiomer. Ketoprofen was taken at a dose of 75 mg by healthy volunteer [28].

The results in the paper indicate that this chiral HPLC method is suitable to quantify directly ketoprofen enantiomers in both human and rat plasma. Separation was performed on a Chiralpak AD column attached to 5 cm Supelcosil LC-SI at constant temperature (30 °C). The minimum quantifiable concentration of the assay was found to be 0.025 or 0.25 μg/ml based on 1 ml human or 0.1 ml of rat plasma samples, respectively. This method provides a similar sensitivity to stereospecific assays previously reported with the advantage of shorter sample preparation and run times [29].

The resolution of racemic tiaprofenic acid in plasma has been performed using immobilized human serum albumin as the stationary phase. The eluent was phosphate buffer-acetronitrile-n-octanoic acid (90 : 10 : 0.015, v/v). Human blood samples were collected after a single ingestion of 200 mg of racemic tiaprofenic acid by two drug-free volunteers. Racemic tiaprofenic acid was prepared in mannitol (10%, 25 mg/ml) before injection. This suspension was administered substaneously to rats at a dose of 5 mg per rat. When the criterium of minimum detectability as three times the system noise was used, the detection limit for each tiaprofenic acid enantiomer was 10 ng in water [30].

A potential problem with chiral derivatization is the possibility of stereochemical conversion during the derivatization reaction. This possibility has been examined with ibuprofen, ketoprofen, flurbiprofen. The plasma samples were analyzed using chiral derivatization with ethyl chloroformate followed by either *R*-(+)-α-phenylathylamine or L-leucinamide [31]. The amide ability to simultaneously separate the enantiomers of 2-arylpropionic acid namely flurbiprofen, ibuprofen and ketoprofen by HPLC in plasma using L-leucinamide as chiral coupling component was described. The derivatives formed by reaction of the carboxylic group of the NSAID with the chiral amine, L-leucinamide, were separated an chiral column in a reversed-phase system. The limit of detection was found to be 0.1 μg/ml for each enantiomer. This HPLC procedure only requires one extraction step of racemates from plasma and a rapid derivatization of less than 3 min. This analytical procedure, employing relatively simple HPLC technology, is sensitive enough for the use in single-dose pharmacokinetic studies [32].

2.3. Plasma and urine

Debleke et al. [33] used HPLC to measure plasma and urine fenoprofen levels in equine biofluids. The detection limits in equine plasma and urine were 0.05 μg/ml (2 ml of plasma) and 0.2 μg/ml (0.5 ml of urine). Ibuprofen in the lower ng range could, up to now, only be determined in biological samples by means of specific detectors. An HPLC assays for the determination of ibuprofen in plasma and urine involved extraction (SPE) of drug. The lower detection limit is 0.5 ng/20 μl of sample volume [34].

The enantiomers of ibuprofen were derivatized with a chiral (*R*)-1-(naphthen-1-yl)ethylamine, using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and 1-hydroxybenzotria-zole as coupling reagents, to yield the corresponding diastereoisomeric amides [35]. An α_1-acid glycoprotein chiral stationary phase has also been demonstrated for the optical resolution of ibuprofen enantiomers in human plasma and urine by HPLC [36].

An isocratic HPLC method has been developed to determine racemic ibuprofen and its major metabolites in biological fluids (human plasma and urine) by means of a conventional reverse-phase column. The method involves the use of a solid phase extraction on "Baker" C18 extraction columns for sample clean-up [37]. Karidas et al. described a simple, extractionales HPLC method with UV detection in human plasma and urine. The method is sufficiently sensitive and fast (total run time is 5 min) for biopharmaceutical studies [38].

Zonal elution and high-performance affinity chromatography were used to study the different binding characteristics of *R*- and *S*-ibuprofen with the protein human serum albumin. Protein binding in blood is a significant factor in the transport and release of many drugs and hormones. These interactions can influence the biological distribution of these compounds as well as their excretion, therapeutic activity and toxicity [39].

The ability to separate ketoprofen enantiomers by HPLC at ambient temperature on commercially available 250∗406 mm amylase carbamate-packed chiral column (Chiralpak AD) column with hexane-isopropyl alcohol-trifluoroacetic acid as the mobile phase without pre-column derivatization has been presented [40]. Ketoprofen enantiomers derivatized with ethylchloroformate *S*-(−)-α-phenyl-ethylamine in triethylamine were detected by HPLC [41].

A validated HPLC method has been published for determining the enantiomers of naproxen and 6-O-desmethyl-naproxen on the second generation Chiral-AGP column in biological fluids. Two rats were each given 20 mg oral doses of (*R*)-naproxen dissolved in 2 ml of 0.1 M potassium phosphate while two other rats were similarly dosed with the *S*-enantiomer. Andersen and Hansen have shown that *N*,*N*-dimethyl octyl amine (DMOA) selectively changes the capacity factor (k') of the (*S*)-form of naproxen. The recovery of the extraction procedure was above 100%. The method has been used for the determination of the optical purity of naproxen. The minimum detectable amount of (*R*)-naproxen in urine from the rats was

0.01 nmol, corresponding to a detection limit of 5 pmol/ml of urine. In all four rats, more than 95% of the dose was extracted in the urine as 6-O-desmethyl naproxen or conjugates of 6-O-desmethyl naproxen. Only the inversion of the (*R*)-form of the naproxen derivative could be detected, which as a further indication of the undirectional nature of the inversion [42]. Naproxen is metabolized in humans by O-demethylation, and by acyl glucuronidation to the 1-O-glucuronide. Naproxen, and phase I and phase II metabolites can be measured directly by gradient HPLC analysis without enzymatic deglucuronidation. A pharmacokinetic profile of naproxen is reported with this method. The limit of quantification of naproxen in plasma is 1.5 μg/ml. The limits of quantification in urine are for naproxen, O-desmethyl-naproxen, naproxen acyl glucuronide and O-desmethyl-naproxen and O-desmethyl-naproxen were all 1.5 μg/ml [43]. A reversed-phase HPLC method based on bare silica, dynamically modified with cetyltrimethylammonium ions, has been suggested and a simultaneous quantitative determination of naproxen, the metabolite 6-O-desmethyl-naproxen and their 5 conjugates in plasma and urine samples has been demonstrated. Two healthy human volunteers were given 250 mg of naproxen orally. Naproxen glycine conjugate and 6-O-desmethyl-naproxen ether-glucuronide could not be detected even after cleavage of the two acyl-glucuronides by 1 M sodium hydroxide at room temperature. The dynamically modified silica approach has already proved to be a valuable separation method in the analysis of biological samples, especially in the simultaneous analysis of the parent drug, metabolites and conjugates, which often is difficult because of the large variation in polarity. The detection limit for 6-O-desmethyl-naproxen O-sulphate with fluorescence detection at 330 nm is 0.5 μg/ml, the limit of detection by UV absorption is 0.1 μg/ml. The detection limit for naproxen acyl-glucuronide, 6-O-desmethyl-naproxen ether-glucuronide, 6-O-desmethyl-naproxen acyl-glucuronide is about 0.1 μg/ml. The limit of quantification for 6-O-desmethyl-naproxen ether-glucuronide and 6-O-desmethyl-naproxen acyl-glucuronide is about 1 μg/ml [44].

A HPLC method for the determination of naproxen, ibuprofen and diclofenac has been reported and assay validation in human plasma and synovial fluid samples was determined. The aim of this study was to determine the plasma synovial fluids drug levels in symptomatic cases of osteo- and rheumatoid arthritis, from serial sample after a minimum period of seven days therapy at ventional dose rates [45].

Parientekhayat et al. measured tiaprofenic acid in infants plasma and urine after a single oral dose by HPLC [46].

3. High performance thin-layer chromatography

A HPTLC method is reported for monitoring plasma concentration of flurbiprofen. A marketed sustained-release capsule formulation of flurbiprofen was evaluated in a single dose bioavailability study in six patient volunteers suffering from mild osteoarthritis. All the subjects received a 200 mg sustained-release capsule of flurbiprofen. The limit of detection for flurbiprofen in plasma was found to be 20 ng. Below this concentration, the spot corresponding to flurbiprofen was not clearly visible. The limit of quantification was 40 ng [47].

HPTLC has been successfully used in the analysis of ibuprofen in plasma. Densitometric evaluation was carried out at 222 nm. Four patient volunteers received a 300 mg sustained-release capsule of ibuprofen. The method combines precipitation and extraction. Although perhaps more time-consuming than simple precipitation, this procedure was considerably more rapid than normal L-L extraction and more thorough than simple precipitation. The limit of detection for ibuprofen was found to be 50 ng in plasma. This was the lowest concentration of ibuprofen in plasma that was accurately detected and integrated by the instrumentation used [48].

4. Liquid chromatography

Development and validation of a LC method for the quantitation of ibuprofen enantiomers in human plasma was reported [49]. In a study presented by Steijger et al. ibuprofen was derivatized with *N*-(4-aminobutyl)-*N*-ethylisoluminol with a carbodiimide method optimized by factorial design analysis. Detection was achieved with the electrochemical reagent generation method and post-column addition of microperoxidase as the catalyst. Ibuprofen was determined in human saliva after oral administration. This requires a sensitive analytical method because ibuprofen is strongly bound to plasma proteins, which means that the concentration of the analyte in saliva is rather low. The analysis of saliva samples offers advantages in comparison with plasma samples because saliva sampling is easy, non-invasive, and stress-free. The limit of quantification was 1.25 ng of ibuprofen per 0.5 ml of saliva, and the limit of detection was 0.7 ng of ibuprofen per 0.5 ml of saliva after an oral dose of 400 mg of ibuprofen [50]. The potential of on-line dialysis as a sample preparation procedure for compounds highly bound to plasma proteins was used for non-steroidal anti-inflammatory drugs as model compounds and column liquid chromatography as the separation technique. A fully automated method was presented for the determination of ketoprofen, ibuprofen, flurbiprofen, fenoprofen and naproxen in human plasma. The limit of detection for ketoprofen and naproxen was 0.1 μg/ml, for fenoprofen 0.5 μg/ml, for ibuprofen 2.0 μg/ml, and for flurbiprofen 0.01 μg/ml [51]. Ibuprofen and naproxen were determined by direct injection of plasma samples into a biocompatibile extraction column. The column is based on particles with a biocompatibile external surface and a hydrophobic internal surface. The pores of the particles are small enough to exclude protein molecules; the drug molecules can penetrate the porous particle and are retained on the hydrophobic internal surface. Biocompatibility of the particles was obtained by reaction of the external surface with the human plasma protein α_1-acid glycoprotein. The surface within the pores of the particles contains hydrophobic C_8 or C_{18} groups. The pores of the silica are small enough to exclude plasma proteins. All isolation methods are time-consuming and introduce errors in the assay. The samples were analysed with the fully automated system. The correlation was 1.000. Manual isolation procedures such as L-L extraction, the use of off-line extraction cartridges or precipitation of plasma proteins followed by centrifugation are very time consuming. These types of isolation procedures also introduce errors in the determination that decrease both accuracy and precision. Therefore, a method that requires a minimum of manipulation of the sample prior to the quantification step is preferred. This is of special importance in large clinical projects where several thousand plasma samples must be analysed with high precision and accuracy [52, 53].

The *in vitro* binding of 2-hydroxy-4-trifluoromethylbenzoic acid to human serum was studied by LC in the pre-

sence of diclofenac, ibuprofen and naproxen [54]. The use of the column liquid-chromatography with diode laser-induced fluorescence detection of naproxen in saliva has been described. The sensitivity of DIO-lif detection illustrated by the $(3-10) \times 10^{-12}$ M detection limit for various derivated carboxylic acids. The real-life detection limit for the carboxylic acid is 3×10^{-8} M, because derivatization is not quantitative at lower concentrations. For this purpose, re-absorbing labels containing hydrazide, bromoacetamide and iodoacetamide were studied [55].

5. Gas-chromatography/mass-spectrometry

5.1. Urine

Toxicological detection of ibuprofen and its metabolites in human urine with GC-MS has been demonstrated by Mauer et al. [56].

5.2. Serum and plasma

GC-MS has been suggested for the determination of ibuprofen in human plasma and compared to analysis by HPLC. The analysis of ibuprofen was sensitive to at least 5 mg/l [57]. The GC assay was capable of quantifying ibuprofen enantiomers in human plasma with optically active *R*(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol as derivatizing reagent and oxalyl chloride as the coupling reagent. GC-MS with selected-ion monitoring was used to determine the efficiency of the separation and for quantification. The minimum quantifiable concentration of each derivatized enantiomer was ca. 5 ng/ml in plasma [58].

A method for the quantitative measurement of ketoprofen in human plasma presented by Leis et al. was based on gas chromatography/negative ion chemical ionization mass spectrometry [59]. The assay was based on gas Taylor describes GC method, developed to measure concentrations of unchanged ximoprofen in the plasma of human subjects. The method was applied to plasma samples obtained from twelve male volunteers after each had ingested a 30 mg single oral dose of ximoprofen contained in gelatine capsules (2×15 mg). Plasma was extracted with diethylether. The limit of detection was 0.04 μg/ml. Studies in patients suffering from various rheumatic diseases have shown that ximoprofen is active at a dose level of 30 mg per day with a low incidence of gastro-intestinal side-effects [60].

A GC method has been reported to study plasma protein binding of ibuprofen and ketoprofen enantiomers by selected-ion monitoring. As these NSAIDs are extensively bound to plasma and synovial fluid proteins (≥99%), the need to measure unbound enantiomer drug levels of the other of 5 ng/ml using 5.0 μl or less of synovial fluids is not uncommon. A single 400 mg dose of *R*,*S*-ibuprofen or 100 mg of *R*,*S*-ketoprofen was given to fasting patients with a glass of water. The detection limits for the ibuprofen enantiomers were less than 1 ng/ml whilst the minimum quantifiable concentration from a 200 μl sample of synovial fluid was 3 ng/ml. The detection limits for the ketoprofen enantiomers were less than 1 ng/ml whilst the minimum quantifiable concentration from a 200 μl sample of synovial fluid was 2 ng/ml. This enhanced sensitivity over previous assays allowed quantification of very low levels (≥25 ng/ml) of unbound enantiomers of these drugs in synovial fluid and plasma [61].

A GC assay capable of resolving enantiomers of NSAIDs is based on a derivatization procedure. The NSAIDs dissolved in triethylamine were silylated with *N*-methyl-*N*-(*tert*.-butyldimethylsilyl)trifluoroacetamide (TBDMS) in isooctane at room temperature for 30 min and subsequently analysed by capillary GC and GC/MS. The TBDMS derivatives were eluted as untailed sharp peaks [62].

6. Capillary electrophoresis

In human urine obtained following administration of ibuprofen, the drug itself, the glucuronide metabolite, the hydroxylated metabolite and its corresponding glucuronide, and the carboxylic acid metabolite as the glucuronide were all rapidly identified by GC-MS [63].

CZE can be utilized for ibuprofen analysis in serum in a borate buffer. Ibuprofen has a wide therapeutic window of 10–50 mg/l and a toxic level of >100 mg/l. It has been shown that high doses, resulting in serum concentrations of 50–100 mg/l, which are close to the toxic level, can decrease plumonary complications in patients with cystic fibrosis. Under these conditions, monitoring serum levels becomes important [64].

CE and HPLC were used separately to determine ketoprofen in serum. In order to detect such low levels (1–5 mg/l), the sample needs to be either concentrated on or outside the capillary. Serum was deprotonized with acetonitrile in the presence of an internal standard to remove serum proteins. The minimum detectable level by CZE was 0.6 mg/ml. In the case of HPLC, the analysis used a CN rather than a C_{18} column to reduce the amount of acetronitrile necessary to elute the drug. The minimum detectable level is 0.1 mg/l. The serum levels of ketoprofen in five samples, obtained 2 h after the oral intake of 25 mg of ketoprofen were in the concentration range of 0.8–2.1 mg/l, indicating a good correlation between CZE and HPLC. In absence of stacking, the peak height by CE would be about 10 times less. This illustrated the importance of stacking brought about by the acetonitrile, which will be helpful for analysis of other drugs. Both CZE and HPLC methods give similar results with comparable simplicity and speed. Because the capillaries are less expensive than the HPLC column, CZE is more cost effective to operate. On the other hand, HPLC has better sensitivity and better reproducibility and is less affected by the sample matrix [65].

Several linear maltodextrin oligosaccharides have been used as potential chiral selectors for the separation of ibuprofen in a blood serum sample by capillary electrophoresis [66].

7. Optical methods

Konstantianos et al. described a method based on the intrinsic fluorescence of naproxen and is main metabolite, the 6-demethylated derivate, in human serum. The limit of detection for naproxen was 0.003 mg/l [67, 68]. The interaction of naproxen with thallium (I) ions in the presence of sodium dodecyl sulphate allows for a rapid determination of naproxen in serum by stopped-flow room-temperature liquid phosphorimetry [69]. Panderi and Parissipolou investigated methods for determinating naproxen in the absence or presence of its 6-desmethyl metabolite in human plasma by a second-derivative spectrophotometric method. The method consists of direct extraction of the nonionized form of the drug with pure diethylether and quantifies naproxen by measuring the peak amplitude in the second-order derivative spectrum at a wavelength of

328 nm. The results obtained by this method were in good agreement with those found by an HPLC method [70].

8. Other methods and drugs

Direct current polarography and cyclic voltammetry were used to study the reduction behaviour of tiaprofenic acid, whereas direct current stripping voltammetry was applied to the quantitative measurement of the drug in dosage forms and in biological media [71].

A new method based on the C13-labeling and NMR spectroscopy was used to study the human urinary excretion of diastereomeric acylglucuronides after oral administration of a racemic [3-C-13] ketoprofen [72].

Alminoprofen and bermoprofen are undergoing clinical trials but there are no assays published in the literature. Benoxaprofen, indoprofen and cicloprofen are no longer used clinically. Miroprofen is no longer used clinically and no assays have been pubished in the literature. No assays for pirprofen and pranoprofen in biological fluids are reported.

9. Conclusions

A resurgance of electrophoretic techniques for drug assays on biofluids is now real. In the CZE mode it may prove very valuable for studing charged metabolites such as glucuronides. CE is well suited for chiral separations of drugs, as demonstrated with standard mixtures and in quality control applications. An important advantage of CE is that it not only needs little sample preparation but it also consumes very little buffer and buffer additives, such as chiral reagents. However, much remains to be done to make CE a robust and useful technique for biofluids. In particular, CE still suffers from a general lack of concentration sensitivity. Some are generally applicable such as increased path length detectors and various means of increasing sample loading such is ITP sample introduction. Specificity can also be achieved by the use of appropriate detectors. Matrix effects need to be minimized by techniques such as isotachophoretic loading or SPE of the sample prior to CE.

CE offers many interesting research possibilities. For instance, bound and free forms of a drug can be assayed simultaneously since the free drug and the protein-bound complex will have different mobilities in appropriate electrolytes.

It can be seen that HPLC and GC are useful analytical techniques, which are capable of separation of arylpropionic acids in biological matrices. This publication has shown that capillary electrophoresis appears to be a valuable method for the determination of NSAIDs in biological fluids. With the increased use of these methods, further advances in analytical methods and their application of NSAIDs in biological fluids can be anticipated.

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