Review Article

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Review of characteristics and analytical methods for determination of indomethacin

Supplementary material

Analytical methods used for indomethacin (INDO) determination between 1965 and 1999 are described below.

S1 Colorimetry

Abdel-hay et al. [1] applied colorimetry for INDO determination in 1990. The method was based on the reaction of INDO with 2-nitrophenylhydrazine in the presence of dichlohexylcarbodiimide in ethanol to obtain a compound of intense violet color with a maximum absorption at 550 nm. The measuring range of the method was 14.31–71.56 $\mu g \cdot mL^{-1}$.

Bourinbaiar and Lee-Huang [2] applied colorimetry for clinical purposes. They investigated the effect of INDO on HIV replication and concluded that INDO could serve as a leading compound for anti-HIV drug design. Although it was only used in a small part of the study (determination of cytotoxicity of INDO), the contribution of colorimetry to the overall result can be seen.

S2 Fluorimetry

The history of the fluorometric determination of INDO started in 1965 when Holt and Hawkins [3] studied the use and absorption of INDO in patients with rheumatoid arthritis, ankylosing spondylitis, and osteoarthritis. They determined INDO in human serum. This paper presents a major shift in the clinical application of INDO determination.

In 1972, Hvidberg et al. [4] modified a method developed by Holt and Hawkins. Their aim was to investigate the binding of INDO to human plasma proteins and its rate of disappearance from plasma after oral administration. In contrast to the Holt and Hawkins study that used NaOH to directly measure fluorescence, their determination was based on measuring the fluorescence of INDO in phosphate buffer (pH 11.6), because they found that fluorescence developed with greater intensity in a less alkaline solution.

In 1986, INDO was determined in a different type of sample (pharmaceutical preparations) using fluorometry for routine pharmaceutical analysis. The analytical procedure was based on the fluorescent reaction of INDO with *m*-aminophenol-chloroamine-T. It was concluded that this method was precise and selective and could be used in routine analyses [5].

S3 Phosphorimetry

In 1997, Arruda and Campiglia [6] developed a simple, rapid, and sensitive solid-surface phosphorimetric method for the determination of INDO in pharmaceutical formulations. For the first time, the phosphorescence of INDO was studied at room-temperature. The INDO hydrolysis reaction in a basic medium was used for the induction of phosphorescence emission, and chromatography paper treated for background reduction was used as a solid substrate.

S4 Gas chromatography

In 1978, Sibeon et al. [7] developed a sensitive and specific gas chromatography (GC) method to determine INDO

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in blood and urine samples. As the GC determination of INDO requires derivation of the substance prior to injection into the gas chromatograph, after extraction of the INDO with ethylene dichloride, pentafluorobenzyl bromide was used for derivative formation.

In 1978, GC was taken to a new level when Møller Jensen [8] developed a method for INDO determination in serum using an extractive alkylation technique and GC. INDO was converted into an anionic form and transferred into a dichloromethane, as an ion pair with a quaternary ammonium ion. When in the dichloromethane, the INDO was converted to an ethyl ester by means of ethyl iodide and determined by GC. The metabolite O-desmethylindomethacin was added to the serum and determined in the same way. The extractive alkylation used in this method does not involve the usual risks, such as the use of explosive and toxic reagents, due to the process of converting the INDO into its ethyl ester.

In 1983, Guissou et al. [9] continued to develop a GC method with electron-capture detection for the determination of INDO in human plasma and urine. Considering that the resulting concentration of O-desmethylindomethacin metabolites may represent an accurate index of INDO biotransformation in humans, authors determined the concentration of the INDO as well as its metabolite. Despite the disadvantage of a long preparation of samples, higher sensitivity was achieved compared to other studies in which the HPLC method was applied.

To improve the main flaws of GC, as listed in the introductory paragraph, Nishioka et al. [10] developed a capillary GC to determine low concentrations of INDO in plasma. They improved the procedures of previous sample treatments by applying solid-phase extraction and ethylation with 1-ethyl-3-*p*-tolyltriazene, which is low in toxicity and stable in diethyl ether for a long period.

In 1990, Dawson et al. [11] improved the method using GC in combination with mass spectrometry for determination of INDO in plasma and synovial fluid. The method was based on negative ion chemical ionization followed by monitoring of fragmentation ion at m/z 312. That fragmentation ion was generated by decarboxylation of the INDO molecular ion at m/z 356. The limit of quantification for this method was 0.1 ng·mL^{-1}.

S5 High performance liquid chromatography

The determination of INDO using high performance liquid chromatography (HPLC) began in 1980 when Terweij-Groen et al. [12] described the quantitative determination of this drug from blood and urine samples. This simple method also enabled the detection of INDO metabolites, which was of great importance for studying the pharmacokinetics of this drug. The authors used ethanol, *n*-butanol, and aqueous buffer as the mobile phase, C_{18}-bonded silica as the stationary phase, and UV detection at 235 nm.

One year later, Plakogiannis et al. [13] developed a sensitive and precise method for the determination of different types of INDO-based drugs in routine pharmaceutical analyses using HPLC. They used acetonitrile–acetic acid (55:45) as the mobile phase, and UV detection at 254 nm.

Bernstein and Evans [14] combined fluorimetry with HPLC to obtain a sensitive and specific method for the determination of INDO and its metabolites in urine and plasma. As the mobile phase, they used 22.5 or 26% acetonitrile in 0.25% acetic acid.

In 1982, Astier and Renat [15] developed an HPLC method with UV detection (250 nm) for the determination of INDO and its metabolites in human plasma at concentrations down to 20 ng·mL^{-1}. Compared to previous research, the preparation was shorter because it involved deprotonation and extraction in just one step. The mobile phase was acetonitrile–acetic acid (60:40). They also used a precolumn and studied the disappearance of two different formulations of INDO in the plasma of subjects.

In 1984, Smith and Benet [16] developed an HPLC method with UV detection (254 nm) to determine the total quantity of INDO (free compound plus glucuronide conjugate), and its deschlorobenzoyl and desmethyl metabolites in urine. As the mobile phase, they used acetonitrile–acetic acid (30:70). The proposed method allowed direct determination of the compounds, and the chromatography had better resolution compared to other studies.

De Zeeuw et al. [17] proposed INDO determination in biological fluids using an HPLC method combined with post-column in-line alkaline hydrolysis, which enabled the detection of fluorescence in the low picogram range. As the mobile phase, they used methanol–water (52:48).

Mawatari et al. [18] developed an HPLC method involving a post-column photochemical reaction and fluorometric detection. INDO was determined in human serum using phosphate buffer (pH 6.6)–acetonitrile (65:35) as the mobile phase. The post-column reaction could be carried out only by UV irradiation.

In 1990, Hubert and Crommen [19] described a sensitive and automatic method for the determination of INDO in human plasma using UV detection at 254 nm. The method was based on liquid–solid extraction on

disposable extraction cartridges before on-line injection into the HPLC. The mobile phase was methanol–phosphate buffer pH 7.4 (60:40). The authors used an autosampler equipped with a robotic arm, with a needle for handling the plasma samples. It can be seen that the development of this very precise and automated method facilitates and accelerates further analyses.

In 1992, Kubo et al. [20] proposed the combination of an HPLC method with fluorometric detection for INDO determination. As the mobile phase the authors used 35% acetonitrile in phosphate buffer (pH 10.0). They introduced post-column alkaline hydrolysis at a high temperature to form a fluorophore. The method was sufficiently specific for the assessment of INDO in serum and could also be used in routine therapies.

In the same year, Johnson and Ray [21] developed a simple HPLC method for INDO determination in plasma samples. They used 80% methanol in anhydrous sodium acetate buffer as the mobile phase, and UV detection at 320 nm. The developed method could be applied in routine laboratory analyses.

A year after the previous research, Vree et al. [22] developed a more complex research, emphasizing the pharmacokinetics and metabolism of INDO in the human body. The authors proposed an accurate gradient HPLC method for the detection of INDO, its metabolite, O-desmethylindomethacin, and their conjugates in plasma and urine samples using UV detection at 254 nm. As the mobile phase, they used acetonitrile–orthophosphoric acid. This method enabled the direct measurement of the analytes without enzymatic deglucuronidation and thus accelerated the overall analysis.

In 1994, Niopas and Mamzoridi [23] developed a simple, sensitive, fast, and reliable HPLC method for pharmacokinetic studies and routine monitoring of INDO in the plasma of children and newborns. The blood samples were deproteinized with acetonitrile and then analyzed using HPLC with UV detection at 280 nm. The mobile phase was phosphoric acid–acetonitrile (40:60). This method was valuable for pediatric use.

In 1997, the HPLC determination of INDO from plasma samples continued, following a very similar principle to previous studies. Once again, the first step of the HPLC method proposed by Sato et al. [24] involved plasma deprotonation with acetonitrile. They also used phosphoric acid–acetonitrile (50:50) as the mobile phase and UV detection (205 nm). In addition to being simple and precise, this method required minimal pre-treatment of samples and was also suitable for monitoring INDO therapy in premature infants.

In 1998, another study with HPLC and UV detection (254 nm) of INDO was published. This time, completely different types of sample were used, as Cristòfol et al. [25] applied an animal sample of chicken tissues (muscle, liver, fat), which had not been applied previously. Three days after treatment, it was concluded that there was no significant concentration of INDO in the tissue. As the mobile phase, the authors used acetonitrile–acetic acid (50:50).

S6 Voltammetry

The determination of INDO by voltammetry began in 1998 when Radi [26] described the electrochemical oxidation and detection of INDO in phosphate buffer (pH 7) on carbon paste electrodes. The author characterized electrodes based on Nujol, silicone, and castor oil pasting liquid and found that INDO could be selectively extracted from aqueous solution into the castor oil electrode. After its accumulation, the INDO was successfully determined in urine samples. This fast, selective, and sensitive method could be applied for multiple determination of INDO.

In 1999, Ali [27] used CV, cathodic stripping voltammetry (CSV), and differential pulse stripping voltammetry (DPSV) for the determination of INDO in urine, serum, and pharmaceutical preparations. The developed methods were based on the adsorptive preconcentration of INDO on a hanging mercury drop electrode (HMDE), followed by tracing the voltammogram in a cathodic potential scan, and the described methods were simple, fast, sensitive, and easily applicable for routine analyses.

S7 Potentiometry

The first ISE for INDO determination was developed by Aubeck et al. [28] in 1991. The electrode was based on the bis(triphenylphosphoranylidene) ammonium-INDO ion pair in a PVC liquid type membrane. They used direct potentiometric measurements for INDO determination in a pure system.

S8 Immunochemical methods

Immunochemical determination of INDO began in 1977, when Hare et al. [29] developed a radioimmunoassay

(RIA) to determine INDO in biological fluids. They produced antibodies in rabbits that were immunized with a conjugate of bovine serum albumin and INDO. The amount of antibody was determined by binding radiolabeled INDO (2-^{14}C-INDO) and antiserum. Diluted antiserum was added to the blood sample of subjects treated with INDO (unlabeled INDO), after which a competitive reaction occurred. They measured the radioactivity using a scintillation counter.

Two years after the first study, Alam et al. [30] developed an RIA based on the use of protein A labelled with ^{125}I, as a tracer, for the quantification of anti-INDO in picogram amounts in biological fluid.

References

[1] Abdel-Hay MH, Korany MA, Bedair MM, Gazy AA. Colorimetric determination of seven nonsteroidal anti-inflammatory drugs using 2-nitrophenylhydrazlne hydrochloride. Anal Lett. 1990;23(2):281–94. doi: 10.1080/00032719008052444.

[2] Bourinbaiar AS, Lee-Huang S. The non-steroidal anti-inflammatory drug, indomethacin, as an inhibitor of HIV replication. FEBS Lett. 1995;360(1):85–8. doi: 10.1016/0014-5793(95)00057-G.

[3] Holt LPJ, Hawkins CF. Indomethacin: studies of absorption and of the use of indomethacin suppositories. Br Med J. 1965;1(5446):1354–6. doi: 10.1136/BMJ.1.5446.1354.

[4] Hvidberg E, Lausen HH, Jansen JA. Indomethacin: plasma concentrations and protein binding in man. Eur J Clin Pharmacol. 1972;4(2):119–24. doi: 10.1007/BF00562508.

[5] Sastry CSP, Mangala DS, Ekambareswara Rao K. Spectrophotometric and fluorimetric methods for the determination of indomethacin. Analyst. 1986;111(3):323–5. doi: 10.1039/an9861100323.

[6] Arruda AF, Campiglia AD. Phosphorimetric determination of indomethacin in pharmaceutical formulations. Analyst. 1997;122(6):559–62. doi: 10.1039/a700309i.

[7] Sibeon RG, Baty JD, Baber N, Chan K, Orme MLE. Quantitative gas-liquid chromatographic method for the determination of indomethacin in biolgical fluids. J Chromatogr A. 1978;153(1):189–94. doi: 10.1016/S0021-9673(00)89871-4.

[8] Jensen KM. Determination of indomethacin in serum by an extractive alkylation technique and gas-liquid chromatography. J Chromatogr A. 1978;153(1):195–202. doi: 10.1016/S0021-9673(00)89872-6.

[9] Guissou P, Cuisinaud G, Sassard J. Gas chromatographic determination of indomethacin and its O-desmethylated metabolite in human plasma and urine. J Chromatogr B Biomed Sci Appl. 1983;277(C):368–73. doi: 10.1016/S0378-4347(00)84860-4.

[10] Nishioka R, Harimoto T, Umeda I, Yamamoto S, Oi N. Improved procedure for determination of indomethacin in plasma by capillary gas chromatography after solid-phase extraction. J Chromatogr B Biomed Sci Appl. 1990;526(C):210–4. doi: 10.1016/S0378-4347(00)82500-1.

[11] Dawson M, Smith MD, McGee CM. Gas chromatography/negative ion chemical ionization/tandem mass spectrometric quantification of indomethacin in plasma and synovial fluid. Biol Mass Spectrom. 1990;19(8):453–8. doi: 10.1002/bms.1200190802.

[12] Terweij-Groen CP, Heemstra S, Kraak JC. Rapid determination of indomethacin and salicylic acid in serum by means of reversed-phase liquid chromatography. J Chromatogr B Biomed Sci Appl. 1980;181(3–4):385–97. doi: 10.1016/S0378-4347(00)81141-X.

[13] Plakogiannis FM, Ali A, Kazmi S. High-pressure liquid chromatographic determination of indomethacin in capsules. Drug Dev Ind Pharm. 1981;7(2):215–21. doi: 10.3109/03639048109057712.

[14] Bernstein MS, Evans MA. High-performance liquid chromatography-fluorescence analysis for indomethacin and metabolites in biological fluids. J Chromatogr B Biomed Sci Appl. 1982;229(1):179–87. doi: 10.1016/S0378-4347(00)86049-1.

[15] Astier A, Renat B. Sensitive high-performance liquid chromatographic determination of indomethacin in human plasma. Pharmacokinetic studies after single oral dose. J Chromatogr B Biomed Sci Appl. 1982;233(1):279–88. doi: 10.1016/S0378-4347(00)81754-5.

[16] Smith PC, Benet LZ. High-performance liquid chromatographic method for the determination of indomethacin and its two primary metabolites in urine. J Chromatogr B Biomed Sci Appl. 1984;306(C):315–21. doi: 10.1016/S0378-4347(00)80893-2.

[17] De Zeeuw D, Leinfelder JL, Craig Brater D. Highly sensitive measurement of indomethacin using a high-performance liquid chromatographic technique combined with post-column in-line hydrolysis. J Chromatogr B Biomed Sci Appl. 1986;380(C):157–62. doi: 10.1016/S0378-4347(00)83637-3.

[18] Mawatari KI, Iinuma F, Watanabe M. Fluorimetric determination of indomethacin in human serum by high-performance liquid chromatography coupled with post-column photochemical reaction with hydrogen peroxide. J Chromatogr B Biomed Sci Appl. 1989;491(C):389–96. doi: 10.1016/S0378-4347(00)82856-X.

[19] Hubert P, Crommen J. Automatic determination of indomethacin in human plasma using liquid-solid extraction on disposable cartridges in combination with HPLC. J Liq Chromatogr. 1990;13(19):3891–907. doi: 10.1080/01483919008049576.

[20] Kubo H, Umiguchi Y, Kinoshita T. Fluorometric determination of indomethacin in serum by high performance liquid chromatography with in-line alkaline hydrolysis. Chromatographia. 1992;33(7–8):321–4. doi: 10.1007/BF02275910.

[21] Johnson AG, Ray JE. Improved high–performance liquid chromatographic method for the determination of indomethacin in plasma. Ther Drug Monit. 1992;14(1):61–5. doi: 10.1097/00007691-199202000-00010.

[22] Vree TB, van den Biggelaar-Martea M, Verwey-van Wissen CPWGM. Determination of indomethacin, its metabolites and their glucuronides in human plasma and urine by means of direct gradient high-performance liquid chromatographic analysis. Preliminary pharmacokinetics and effect of probenecid. J Chromatogr B Biomed Sci Appl. 1993;616(2):271–82. doi: 10.1016/0378-4347(93)80395-K.

[23] Niopas I, Mamzoridi K. Determination of indomethacin and mefenamic acid in plasma by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl. 1994;656(2):447–50. doi: 10.1016/0378-4347(94)00116-2.

[24] Sato J, Amizuka T, Niida Y, Umetsu M, Ito K. Simple, rapid and sensitive method for the determination of indomethacin in plasma by high-performance liquid chromatography with ultraviolet detection. J Chromatogr B Biomed Appl. 1997;692(1):241–4. doi: 10.1016/S0378-4347(96)00463-X.

[25] Cristòfol C, Pérez B, Pons M, Valladares JE, Martí G, Arboix M. Determination of indomethacin residues in poultry by high-performance liquid chromatography. J Chromatogr B Biomed Appl. 1998;709(2):310–4. doi: 10.1016/S0378-4347(98)00083-8.

[26] Radi A. Preconcentration and voltammetric determination of indomethacin at carbon paste electrodes. Electroanalysis. 1998;10(2):103–6. doi: 10.1002/(SICI)1521-4109(199802)10:2<103:AID-ELAN103>3.0.CO;2-R.

[27] Ali AMM. Cathodic adsorptive stripping voltammetric determination of the anti-inflammatory drug indomethacin. J Pharm Biomed Anal. 1999;18(6):1005–12. doi: 10.1016/S0731-7085(98)00111-3.

[28] Aubeck R, Bräuchle C, Hampp N. Indomethacin ion-selective electrode based on a bis(triphenylphosphoranylidene)ammonium-indomethacin complex. Analyst. 1991;116(8):811–4. doi: 10.1039/AN9911600811.

[29] Hare LE, Ditzler CA, Duggan DE. Radioimmunoassay of indomethacin in biological fluids. J Pharm Sci. 1977;66(4):486–9. doi: 10.1002/jps.2600660407.

[30] Alam I, Levine L, Langone JJ. Immunoassay of indomethacin: the use of [^{125}I] protein A to detect specific serologic binding. Prostaglandines Med. 1979;2(3):167–75. doi: 10.1016/0161-4630(79)90034-X.