Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(4):1591-1596

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

New assay method UV spectroscopy for determination of Indomethacin in pharmaceutical formulation

Karima Fadhil Ali, Ali Rasool Mahmood Albakaa and Zinah Hussein Ali

Dept. of Pharmaceutical Chemistry, College of Pharmacy, University of Al-Mustansiriyah, Baghdad, Iraq

---

ABSTRACT

New, simple and rapid method indicating UV spectroscopy was developed and validated for the estimation of Indomethacin(IND)in pure form, and in respective formulations. (0.1N) KOH solution was used as a solvent to decompose IND to p-Chlorobenzoic acid and 5-methoxy-2-methyl-3-indoleacetic acid .The adequate drug solubility and maximum assay sensitivity was found asp-Chlorobenzoic acid, the maximum absorbance of p-Chlorobenzoic acid was measured at (228nm)in the wavelength range of (200-650 nm), the linear calibration curve was obeyed in the concentration range of (1-10 ppm)show regression equation (Y =0.1263X -0.002), and correlation Coefficient (R^2=0.9949). This method was validated and applied to the determination of IND in capsules from different companies in Iraqi market, no interference was found from capsule excipients at the selected wavelength and analysis conditions. It was concluded that the developed method is accurate, sensitive, precise, and reproducible; as well as itcan be applied directly for the estimation of p-Chlorobenzoic acid and indirectly for the estimation of IND content in pharmaceutical formulations.

Key words: UV Spectroscopy, Indomethacin (IND), p-Chlorobenzoic acid, Calibration curve method.

INTRODUCTION

Indomethacin is a nonsteroidal, anti-inflammatory agent with antipyretic, analgesic properties and is an indole derivative designated chemically as 1-(p-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid. IND is an odorless, pale yellow to yellow tan crystalline substance. It is lipid-soluble, practically insoluble in water and sparingly soluble in alcohol. IND has a pKa of 4.5 and is stable in neutral or slightly acidic media and decomposes in strong alkali. The suspension has a pH of 4.0–5.0 and it has a melting point between 155°C and 161°C and has molecular weight of 357.8. IND has a molecular formula of $C_{18}H_{16}ClN0_4$[1]

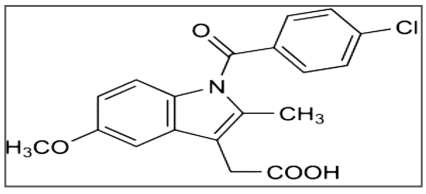

Fig. 1 Chemical structure of indomethacin

Indomethacin can exist in several polymorphic solid forms and as an amorphous solid. The polymorphism is believed to arise from different orientations between the aromatic indole and phenyl rings[2]The measured solubility can change with over time.[3]Suggesting that there is conversion from one form to another. The used of CheqSol approach[21] to reinvestigate the aqueous solubility of indomethacin also found evidence suggesting an amorphous form, as well as evidence to suggest that indomethacin might have decomposed during the measurement reported in [4] the decomposition, which occurs at high pH.

It can be identified by using UV spectroscopy in the range of(215-330nm) wavelength depending on the type of solvent. Several analytical methods have been employed for the assaying IND in pure as well as in pharmaceutical dosage forms, these methods include colorimeter[5], HPLC-UV[6], diazotized *p*-phenylenediamine dihydrochloride[7], electro-oxidation[8], nano-particles[9], partial least square method[10], dimethylcinnam-aldehyde in H_2SO_4[11], LC-MS[12], 2-nitrophenyl-hydrazine[13], polarography[14], spectrophotometer methods using Ehrlich's reagent[15], *m*-aminophenol-chloramine-T[16], phosphorimetric method[17] densitometry[18], and $NaNO_2$ in H_2SO_4[19]. The official (BP) method[20] for the IND pure form is assayed by titration in acetone using NaOH, while the capsules and suppositories were assayed by a UV-VIS spectrophotometric method, many of these methods already described for the assay of IND require prior hydrolysis, extensive extraction, heating, and all of these compromised accuracy gave a higher Beer's law range.

Because IND decomposes rapidly at pH ≥12, this invalidates measurements of its solubility that involved any exposure to high pH conditions, and illustrates the importance of selecting appropriate assay conditions when analyzing acid- or base-labile molecules using titration methods. According large mean molecular charge values should be investigated, as they may suggest the occurrence of decomposition. It is shown that in some cases CheqSol assays can be carried out successfully even for pH-unstable compounds if mild starting conditions are utilized[21] .

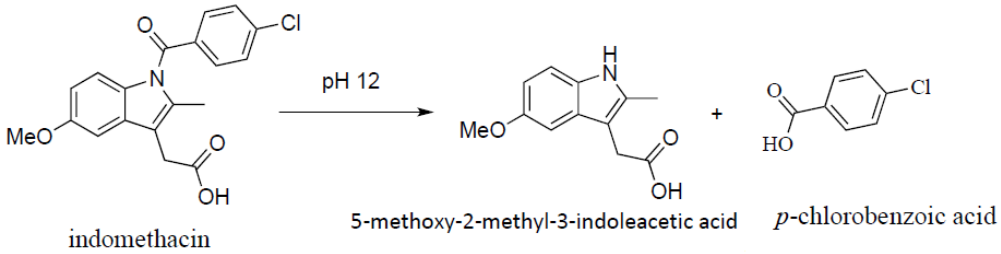

Fig.2.Decompositionof IND into 5-methoxy-2-methyl-3-indoleacetic acid and *p*-Chlorobenzoic acid at pH ≥12

In this study(0.1N) KOH solution was used as a solvent to decompose IND to *p*-Chlorobenzoic acid and 5-methoxy-2-methyl-3-indoleacetic acid (show in fig.2); and assay of IND as *p*-Chlorobenzoic acid. Pure (IND) and IND capsules from six different pharmaceutical companies have been used, which was dissolved in KOH (0.1N)solution and absorbance was measured at a wavelength of (228 nm).The analytical method described was validated and also applied to monitor hydrolysis of IND in alkaline media[22]by standard curve method, to conduct a comparative study of the content of IND from different pharmaceutical companies available in Iraqi pharmaceutical market and to measure the percentage prescribed by the constitution of the drugs approved .

This study was fast, simple, reliable, selective, sensitive and inexpensive UV Spectroscopy method for the determination of IND in commercial pharmaceutical formulations as capsules.

EXPERIMENTAL SECTION

Chemicals and Reagents:

IND RS was used as standard(assigned purity 99.8%), was obtained from the S.D.I. (Iraq), Potassium hydroxide, the capsules containing (25mg) of IND were obtained commercially from the Iraqi market (Table 1), and all other chemical reagents were of analytical grade.

Instrumentation and UV Spectroscopy conditions:

UV-Vis. Spectroscopy (Shimadzu 1800), 160 digital double - beam recording spectrometer, and 1cm Quartz cells; the measurement properties wavelength range (200 – 650) nm, scan speed: medium, sampling interval: 2.0, auto

sampling interval: disabled, scan mode: single, measuring mode: absorbance, slit width: 2.0 nm, light source change wavelength 340.8 nm, S/R exchange: normal, and software name: UV Probe, digital electric balance and micropipette.

Standard stock and standard solutions preparation:
Stock solution (0.250g.L^{-1}) of IND was prepared by dissolving(25mg) of pure IND with KOH(0.1N) in(100 mL) volumetric flask, then diluting with KOH (0.1N) to the mark. Six different standard solutions (2.5-6.375µg.mL^{-1}) were prepared by transferring (0.500-1.275 mL) of stock solution into (50mL) volumetric flasks by micropipette, then diluting with KOH (0.1N) to the mark; the series of standard solutions was shown in (Table.2).

Sample solutions preparation:
IND capsules were used from different companies in the Iraqi market shown in (Table1), twenty capsules of each source of drugs were weighed, an average amount of equivalent to (25mg) of IND dissolved with KOH (0.1N) in (100 mL) volumetric flask, then diluting with KOH (0.1N) to the mark (sample solution).

Table 1: IND (Indocin) 25 mg capsules in the Iraqi market

No	Trade name	Company	M.D.	E.D.	Batch No.	⁎Average Caps Wt.(gm)
1	INDYON 25	MEDOCHEMIE LTD–CYPRUS (EUROPE)	12/2012	12/ 2018	----	0.17128
2	INDOMETHACIN 25mg	TROGE MEDICAL GMBH (Germany)	8/2011	8/ 2015	10718-01	0.14751
3	INDOSAM-25	S.D.I. IRAQ	1/2012	1/ 2017	-----	0.17486
4	Indogesic 25	Dar Al- Dawa (Jordan)	2/2010	2/ 2015	LOT 356F	0.22602
5	INDOFLAM-25	Ajantapharma Limited (India)	10/2012	9/ 2019	C0822J	0.28706
6	Indomethacin 25mg	Beistol Laboratories Ltd. (U.K.)	10/2007	10/2016	----	0.2008

⁎*Average weight twenty capsules*

Then for assay, six solutions prepared from sample solution by transferring (0.600mL) of sample to (50.0mL) volumetric flask by micropipette, then diluted with KOH (0.1N) to the mark (≈3.0µg.mL^{-1}).

Absorbance spectrum of IND:
Transfer (0.700mL) of stock solution (0.250g.L^{-1}) by micropipette up to (50mL) volumetric flask, then diluted with KOH (0.1N) to the mark (3.5µg.mL^{-1}), the absorbance of the solution measured at range (200-650 nm), show the absorbance spectrum andλ_{max} at (228nm)as in Fig.3.

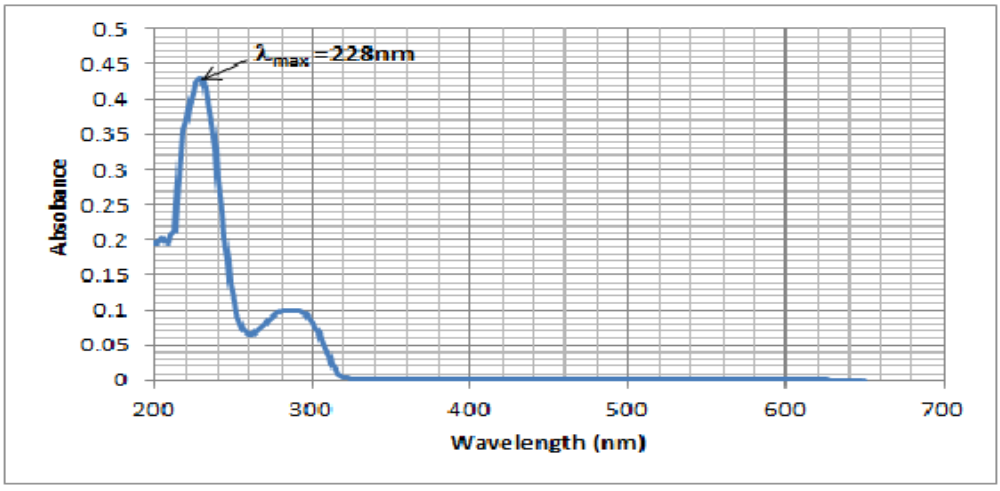

Fig. 3 Absorbance spectrum of IND (λ_{max}228 nm)

Calibration curve:
The method was determined at six concentration levels ranging (2.5-6.5 µg/mL) for IND, the calibration curve was constructed by plotting absorbance versus concentrations of IND (µg/mL), and the regression equation was calculated, each absorbance was the average of six determinations, linear absorbance data for calibration curve was shown in Table.2, and .Fig.4**:**

Table.2: Standard solutions (Concentration and Absorbance)

Concentration ($mg.L^{-1}$)	Absorbance	Notes
0.000	-0.001	y = 0.1263 x - 0.002 Correlation Coefficient R^2 = 0.9949 Chi Square = 0.00927 Standard Error of Estimate = 0.019748 Residual Standard Deviation = 0.02794 Multiple Correlation Coefficient R^2 = 0.99254 Slope= 0.128778 Intercept = - 0.01358
2.550	0.325	
3.188	0.384	
3.825	0.469	
4.681	0.566	
5.100	0.638	
6.375	0.837	

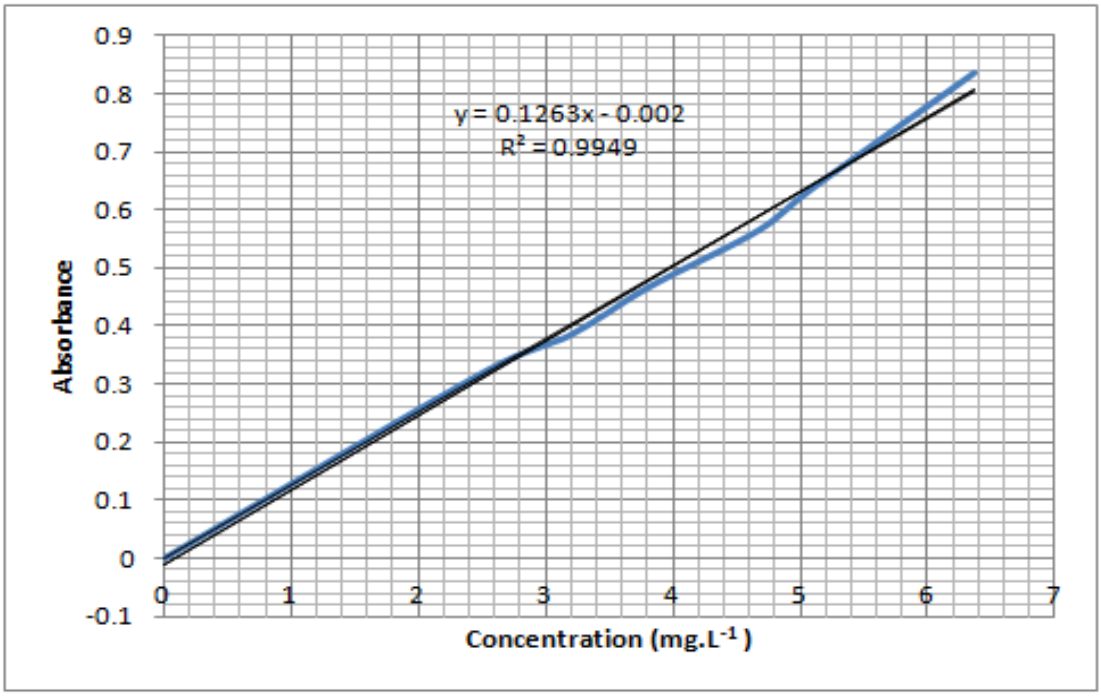

Fig. 4 Calibration curve

RESULTS AND DISCUSSION

Linearity:

The linearity of the drug was obtained for (1-10ppm) concentration range of IND, the regression analysis was performed for line equation, the linear equation was found to be (y = 0.1263 x - 0.002) and correlation coefficient (R^2) 0.9949 (Table.2), the calibration curve was found to be linear in the above stated concentration, and Slope (0.128778), Intercept (- 0.01358), Chi Square (0.00927), Standard Error of Estimate (0.019748), Residual Standard Deviation (0.02794), and Multiple Correlation Coefficient R^2(0.99254).

Precision and Accuracy:

The precision of the analytical system was investigated by performing six consecutive replicate solution of the same standard solution, the standard deviation (SD) and relative standard deviation (RSD) obtained are listed in (Table3) , the low RSD values indicated that the method is precise.

The accuracy of the method (recovery) was investigated by determination of in IND, a solution containing IND (C = 2.55-6.375 mg/L) with no detectable impurities was spiked with the reference substances at appropriate concentrations, the recovery and relative standard deviations (RSD) obtained are listed (Table.3) confirmed the satisfactory accuracy of the method.

Table.3: Precision and accuracy

Concentration of IND (ppm)		Recovery* %	SD	R.S.D* %	Error* %	LOQ	LOD
Taken	Found*						
2.55	2.5141	99.804	0.0235	0.921	-1.408	1.825	0.602
3.188	3.1885	100.016	0.0050	0.157	0.016	0.390	0.129
3.825	3.8245	99.987	0.0062	0.163	-0.013	0.484	0.160
4.681	4.6831	100.046	0.0062	0.133	0.046	0.483	0.160
5.100	5.0816	99.997	0.0093	0.183	-0.003	0.726	0.240
6.375	6.389	100.222	0.0147	0.231	0.222	1.147	0.379

** Six consecutive replicate solution of standard solution.*

Limit of quantification (LOQ), and limit of detection (LOD)

The LOQ and LOD were calculated from the standard deviation (SD) of responses and slope (m= 0.12849),as shown in table 3, the LOQ for IND was up to(1.825- 0.390mg.L^{-1}), while LOD were (0.602- 0.129mg.L^{-1}) as calculated by the below equations:

LOQ = 10(SD/m)

LOD = 3.3 (SD/m)

Method validation:

The method was validated through linearity, sensitivity, precision and accuracy, as shown in Table.4.

The results were checked by UV-Vis. spectroscopy method measure the absorbance at (318.4 nm)of the solution IND diluting with(9:1) methanol: hydrochloric acid(0.1N) solvent[18] the results are in good agreement with this method.

Table.4: Optical characteristics, parameters of the method

No.	Parameters	Result
1	Absorption maxima(λ_{max})	228 nm
2	Linear equation	Y = 0.1263 x – 0.002
3	Regression co-efficient	0.9947
4	Linearity	1-10 mg/L
5	LOD (mg/L)	0.602- 0.129
6	LOQ (mg/L)	1.825 - 0.390

Determination of active IND capsules in the Iraqi market:

This validated method was used to analyze commercially available different brands of IND capsules manufactured by six different companies in Iraqi market. The results revealed that the six marketed brands are compliance with the amount requirement (90-110%) with respect to the labeled claim in Table.5, it was found that IND from TROGE MEDICAL GMBH company had higher Wt.% than others while Ajanta pharma Limited company had lower concentration, in which concentration of each absorbance was determined for six sample solutions of each samples compared to the standard calibration curve in this method, then calculate recovery percent and standard deviation, the validity of the method could be proved by analyzing authentic samples of the drug

Table.5: Determination of IND from Indomethacin capsules companies

No	Trade name	Company	Found*(mg/L)	SD	Wt.%
1	INDYON 25	MEDOCHEMIE LTD–CYPRUS (EUROPE)	2.982	0.0673	99.4%
2	INDOMETHACIN 25mg	TROGE-MEDICAL GMBH (Germany)	3.291	0.0890	109.7%
3	INDOSAM-25	S.D.I. IRAQ	2.736	0.0808	91.2%
4	Indogesic 25	Dar Al Dawa (Jordon)	2.755	0.0389	91.83%
5	INDOFLAM-25	Ajantapharma Limited (India)	2.674	0.0676	89.13%
6	Indomethacin 25mg	Beistol Laboratories Ltd. (U.K.)	2.712	0.0816	90.4%

** Average of six consecutive replicate solution of sample solution.*

CONCLUSION

The successful developed method UV spectroscopy was simple, cheap, effective, rapid, and accurate, with high accuracy and precision values, the validation parameters were evaluated as per Iraqi Ministry of Health guidelines, the satisfactory finding of this work indicates that this analysis technical might be applied for quantitative estimation of IND from pharmaceutical dosage forms, also this method may be employed in routine quality control aspects.

When this method applied for quantitative estimation of IND capsules in Iraqi markets, the results indicate that IND capsules is accepted within the normal percentage (90-110%) according U.S.P.30[23] and Iraqi Ministry of Health.

REFERENCES

[1] M. Brien; J. McCauley; and E. Cohen; K. Florey., Academic Press, London, UK., **2005**, Ed., pp. 211–238,
[2] C. Aubrey-Medendorp; M. J**.** Swadley; T. Li**.,** Pharm. Res., **2008**, 25(4), 953-959
[3] S. B. Murdande; M. J. Pikal; R. M. Shanker; R. H. Bogner., *J. Pharm. Sci*., **2010**, 99(3), 1254-1264.
[4] A. J. Hopfinger; E. X. Esposito; A. Llinàs, R. C. Glen. J. M. Goodman., *J. Chem. Inf. Model*., **2009**, 49(1), 1-5.
[5] O. A. Adegoke *et al.*, *Acta Pharm.*, **2006**, 56, 189–202.
[6] B. Tsvetkova; I. Pencheva; Alexander Z; and P. Peikov, *Int. J. Pharm. Sci.*, **2012**, 4(l3), 549-552.
[7] P. Nagaraja; R. A. Vasantha; and H. S. Yathirajan., *J. Pharm. Biomed. Anal.*, **2003**, 31, 563–569.
[8] Sanjeevaraddi R. Sataraddi; Shreekant M. Patil; Atmanand M. Bagoji; Vijay P. Pattar; and Sharanappa T. Nandibewoor., *ISRN Analytical Chemistry*.,**2014**, Volume 2014 Article ID 816012, 9 pages.
[9] Rezaei Mokarram A; Kebriaee zadeh A; Keshavarz M; Ahmadi A; and Mohtat B., *DARU.*,**2010**. 18(3).
[10] Fouzia Noreen; Tehseen Aman; Amina Mumtaz; and Saima Nazir., *Proc. Pakistan Acad. Sci.*, **2007**, 44(3), 173-179.
[11] C. S. P. Sastry; D. S. Mangala; and K. E. Rao., *Acta Cienc. Indica*., **1986**,126 17–19.
[12] M. E. Abdel-Hamid; L. Novotny; and H. Hamza., *J. Pharm. Biomed. Anal.*, **2001**, 24, 587–594.
[13] M. H. Abdel-Hay; M. A. Korany; M. M. Bedair; and A. A. Gazy. *Anal. Lett.*, **1990**, 23, 281–294.
[14] J. Arcos; J. Lopez-Palacios; and P. Sanchez-Batanero., *Electroanalysis*., **1989**, 1, 279–282.
[15] T. R. Baggi; S. N. Mahajan; and G. R. Rao., *Ind. J. Pharm. Sci*., **1978**, 40, 71–72.
[16] C. S. P. Sastry; D. S. Mangala; and K. E. Rao., *Analyst.*, **1986**, 111, 323–325.
[17] A. F. Arrunda ; A.D. Capiglia., *Analyst*., **1997,**122, 559–562.
[18] J. Krzek; M. Starek., *J. AOAC Int.*, **2001**, 84, 1703–1707.
[19] K. P. R. Chowdary; G. N. Rao; V. B. Rao., *Indian Drugs.*, **1987**, 24, 361–362.
[20] British Pharmacopoeia, Her Majesty's Stationery Office, London **2002**.
[21] John Comer; Sam Judge1; Darren Matthews; Louise Towes; Bruno Falcone2; Jonathan Goodman; John Dearden., ADMET & DMPK., **2014**,2(1),18-32; doi: 10.5599/admet.2.1.33
[22] Sunisha Kulkarni; Surya Prakash Gupta; Neeraj Upmanyu; S.D. Tonpay., *International Journal of Drug Delivery*, **2011** ,3, 141-148.
[23] USP 30- NF25, **2007.**