Determination of Diclofenac Sodium in Pharmaceutical Preparations by Computational Image Scanning Densitometry

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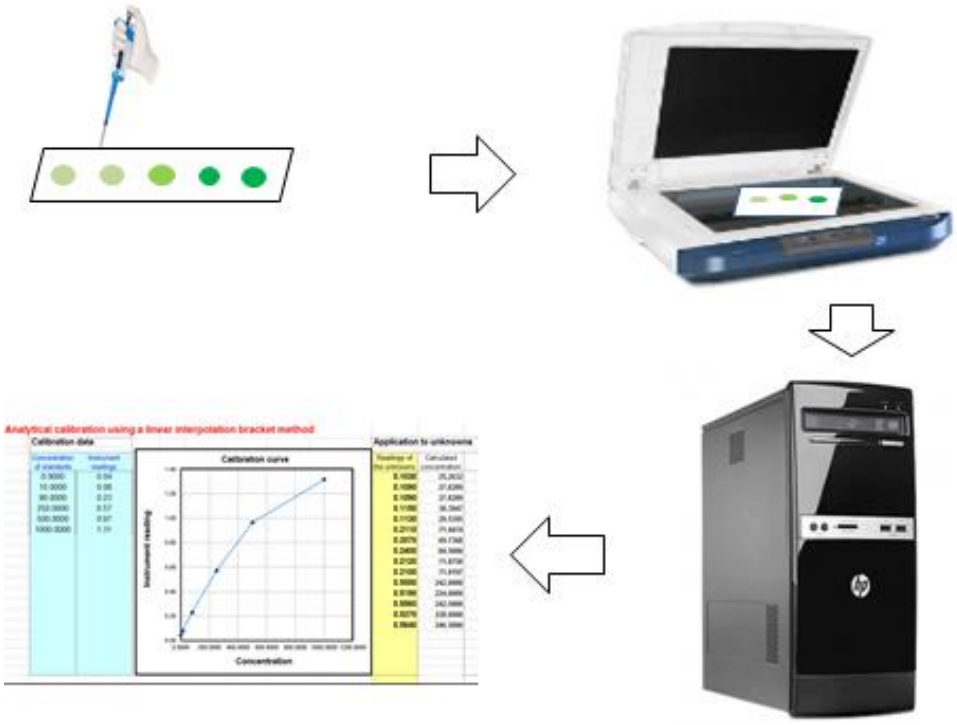
Abstract: Diclofenac sodium is a nonsteroidal anti-inflammatory drug which cures by reducing substances in the body that cause pain and inflammation. There is always a risk of heart attack and stroke in case of taking excess of the drug. In present study a simple, fast and cost-effective method is devised for the assay of diclofenac sodium in locally available pharmaceutical preparations. The method is based on the reaction of the drug with 2, 4-DNPH (2, 4-Dinitro phenyl hydrazine). Micro-quantities of the drug gave green coloured spots when mixed with 2,4-DNPH on a pre-coated TLC plate, in the presence of potassium iodate and lithium hydroxide. The spots were scanned by using a flatbed scanner and the images obtained were computationally quantified with the help of custom-made software to measure the optical density. The reaction parameters were optimized, and the results were compared with the standard spectrophotometric method.



Key Words: Computational Quantification; Image Scanning densitometry; Assay of Diclofenac sodium; Drug Analysis.

Introduction

Diclofenac sodium or Sodium-2-[(2,6-dichlorophenyl)amino]phenyl] acetate is a non-steroidal anti-inflammatory drug which is universally used as pain reliever in rheumatoid arthritis, osteoarthritis, ankyllosing spondylitis, sport injuries and inflammation in human body [1]. It is usually available as a sodium or potassium salt. The sodium or potassium salts of diclofenac are soluble in water and can be easily administered orally. The drug is also used for the treatment of certain inflammatory disorders and

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painful conditions in case of gout, bursitis, headache and menstruation. The sodium or potassium salts of diclofenac are effective in the relief of pain and fever [2]. Alok and co-workers described that the pain-relieving mechanism of DS (diclofenac Sodium) is related to its role of inhibition of the conversion of arachidonic acid to prostaglandins, which are the mediators of the inflammatory process. As compared to other non-steroidal anti-inflammatory drugs used for rheumatoid arthritis and osteoarthritis particularly, DS has far less gastrointestinal effects [3].

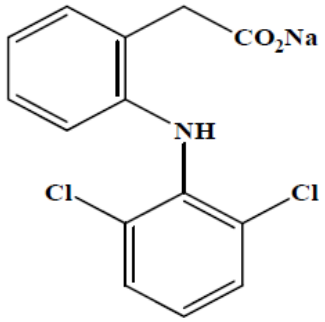

Fig. 1: Sodium salt of diclofenac.

Several analytical techniques have been reported in literature for the assay of diclofenac sodium in pharmaceutical preprations. These techniques include gravimetry [4], potentiometry [5], fluorimetry [6], HPLC [7,8], Flow Injection Anaysis [9], and UV/VIS spectrophotometry [10-13]. Various other techniques like developing sensors for determination of diclofenac sodium have been worked out [14,15] but as being simple, sensitive and fast the spectrophotometric methods are usually prefered over the other techniques used for the determination of the drug. In addition to their adequate precision and accuracy, spectrophotometric methods offer significant practical and economical advantages over other techniques. In most of the spectrophotometric procedures the drug is mixed with a colouring reagent such as Methylene Blue, Methylene Violet, Copper (II) acetate, Iodine, 2,3-dichloro-5,6-dicyanol, 2,4-dichloro-6-nitro-phenol and ferric chloride / 2,2-bipyridine to yield a sufficiently coloured product which could be extracted into an organic solvent [16-18]. Alternatively diclofenac sodium is oxidized with some oxidizing reagent like potassium bromate, ceric ammonium sulfate, dibromodimethylhydantoin or potassium ferricyanide [19-21]. In each case the absorption of the final coloured product is measured at a certain wavelength and employed as a measure of the drug concentration.

Spectrophotometry is although a verstile analytical technique which has been worldwide used for the quantitative analysis of inorganic ions, organic compounds and pharmaceutical products with adequate sensitivity and precision but still it has some limitations. For example spectrophotometry fails in case of precipitation and microlitre samples. It needs a pair of cuvetts and a spectrophotometer to measure the absorption of clear and transparent solution. Similarly spot testing is a very useful and important classical technique in the field of chemical analysis but it also lacks accurate quantification. By combining the positive points of spot testing and spectrophotometry our group developed a novel technique "Computational Image Scanning Densitometry". In this technique a set of coloured spots is developed on a TLC plate by treating the varying concentrations of analyte with colouring reagent and measuring the colour density of each spot on a sanner and a computer with the help of a custom made software. A calibration graph is obtained by plotting the colour density of spots and the concentration of the analyte. By using this technique we determined a number of metal ions [22,23], anions [24,25] and formaldehyde [26]. In this work we used this technique for the estimation of diclofenac sodium in pharmaceutical preprations using a procedure described in literature [27]. The results obtained with CISD (Computational Image Scanning Densitometry) were compared with standard spectrophotometric method.

Experimental

Chemical Reagents

All the chemicals used in this work were of AnalaR grade. The standard solutions were prepared by dissolving the reagents in doubly distilled water. Pure diclofenac sodium was obtained from Sigma-Aldrich Company. Standard stock solution of DS (diclofenac sodium), 1000 µg/mL, was prepared by dissolving 0.1 g of DS in 100 mL pure methanol. Standard solutions of required concentrations (5-50 µg/mL) were prepared by appropriate dilution of stock solution with 50:50 methanol. A solution of 2,4-DNPH (2,4-Dinitrophenyl hydrazine), 0.08 % w/v , was prepared by weighing 0.08 g of the reagent and dissolving it in 2 mL concentrated sulphuric acid and diluting it with 100 mL of distilled water. Potassium iodate solution, 0.4%, was prepared by dissolving 0.4g salt in water and diluting to 100mL. Lithium Hydroxide solution (2N) was prepared by dissolving 4.8 g of the salt in 100 mL distilled water.

Apparatus and Instrumentation

A one mL graduated Micro pipette (Pipettman) and TLC plates (MERCK, TLC, Aluminum Sheets, 20x20 cm, Silica Gel 60 F254) were employed for the development of the spots. A HP 3670 CCD reflective flatbed color scanner was used for digitizing the image of the medium. A VB6 based graphical application with picture box, selection marquee, and flex grid control was developed to measure the color density of the spots from a digital image. A UV-Vis Jenway 6300 Spectrophotometer was used to measure the absorption in standard reference procedure.

Preparation of Pharmaceutical Samples:

Tablets and ampules of different brands manufactured by various pharmaceutical companies containing the drug were obtained from local market. Ten tablets of each brand were weighed and converted into fine powder with the help of an agate pestle and mortar. After thoroughly mixing average weight was calculated and an amount equivalent to 0.1 g of pure DS was taken and transferred to a 100 mL volumetric flask. It was dissolved in a little amount of methanol and then diluted to the mark with 50:50 methanol-water mixture.

Injectable ampoules from two companies labeled as Dicloran and Voren, obtained from local market, were also assayed in this work. Each ampoule contained 3mL drug in liquid form. Half mL liquid from each ampoule was carefully measured with the help of a micro-pipette and transferred into 100 mL volumetric flask. The liquid was diluted to the mark with 50:50 methanol-water mixture.

General Procedure

To react with diclofenac sodium and give a green product as described [24], dizonium cation was prepared in a 5mL flask by mixing 1.5 mL of 0.08 % (m/v) 2,4-DNPH solution and 1.5 mL of 0.15 % (m/v) potassium iodate solution. With the help of a micro pippte 10µL of this mixture was applied on a TLC plate (3x5cm) in the form of ten spots separated by a suitable distance. To react this dizonium cation with the varying quantities of the drug, ten aliquots (each of 10µL) of DS (containing 10 to 100µg/mL of the drug) was added to each spot. After a little drying each spot was soaked with 10µL of 2N lithium hydroxide solution. After a few minutes the spots on the TLC plate turned green with varying intensity of the colour. The same procedure was applied to each commercial sample of the drug. The TLC plate was scanned on a flat bed scanner and the resulting image was analyzed in software to measure the color density of each spot. The calibration graph was plotted between the concentration of the drug and the digitilized color density. The calibration data was used then to find the concentration of the drug in the samples. In order to check the validity of the new method the commercial samples were also analyzed by a standard spectrophotometric method [26] and the results were compared.

Results and Discussion

Spectrophotometry is one of the most versatile, relatively simple and cost effective technique in the field of chemical analysis that is equally useful for quantitative estimation of metal ions, inorganic anions, organic compounds and molecular species which can produce some intensely coloured substance as a result of some chemical reaction such as oxidation, reduction, complexation or some dye formation. The absorption of coloured solution, either aqueous or extracted into some organic solvent, is measured at a certain wavelength and used as a measure of analyte concentration. The versatility of spectrophotometry lies in the chemistry involved to give a coloured product of sufficiently high molar absorptivity. In spite of all the positive aspects of the technique the spectrophotometry still has some limitation where it fails to be employed successfully. For example it needs a certain quantity of analyte which could yield reliable results. It cannot be applied if the sample is already in micro quantities. Secondly the final coloured product should be in form of clear transparent solution absorption of which could be measured at a certain wavelength. Thirdly a reliable and accurately calibrated spectrophotometer is required to measure the absorbance. On the other hand spot tests can be employed for the detection of a large variety of metals, inorganic anions, organic compounds, biological species and environmental pollutants. In spot tests micro quantities of the sample are made to react with a colouring reagent on a suitable substrate like filter paper or a TLC plate resulting a coloured spot. The colour of the spot indicates the presence of analyte. The detection limits for most of the testing species are at sub-micro gram level. The technique is equally useful for coloured solutions as well as coloured precipitates. The major drawback of spot tests is that their use is only limited for qualitative analysis. No reliable technique was available for their accurate quantification. Quantification of various species at micro levels especially in environmental samples is a very crucial requirement. Attempts around the world are going on to find out some cheap, portable and accurate method to determine the desired

content of the sample at the micro level. By developing a novel technique "Computational Image Scanning Densitometry" we combined the positive features of spectrophotometry and spot testing. Coloured spots are developed on a substrate by reacting the analyte with a colouring reagent and their intensity is measured with the help of software loaded on a computer. This practice allowed the determination of micro quantities of a number of substances with adequate accuracy and precision. The major plus point of the technique is that it does not require any conventional instrument and it is easy to handle. It can be employed for the quantification of various species at very low levels with adequate precision. Other advantages include use of extremely small volumes; the coloured precipitation can be quantified; high sensitivity; and sufficient accuracy and precision. In this work also diclofenac sodium was reacted with a dizonium cation resulted by the action of 2,4-DNPH and potassium iodate giving a green spot in alkaline media. The spots were scanned on a flatbed scanner and intensity of each spot was measured with the help of the software loaded in a computer.

Effect of mixing order of the reagent

Experiments performed to check this effect revealed that order of mixing the reagents was very crucial for producing green coloured spot. All the three reagents 2,4-DNPH, potassium iodate and lithium hydroxide were mixed with diclofenac sodium in different orders. Different sequences produced green spots of different intensities except when lithium hydroxide was applied first on DS followed by 2,4-DNPH and potassium iodate respectively. This order did not produce any colour on TLC plate. Maximum colour was produced when an equi-volume mixture of 0.08% 2,4-DNPH and 0.15% of potassium iodate was applied to the DS sample followed by 2N lithium hydroxide. The colour intensities obtained by employing different orders are revealed in Fig. 3.

Effect of concentration of reagents

In order to get the spots of maximum colour intensity effect of concentration of reagents used, 2,4-DNPH, potassium iodate and lithium hydroxide was checked. Keeping the above order of mixing concentration of the reagents was varied. The concentration of 2,4-DNPH was changed from 0.02% to 0.1%. It was observed that 0.08% solution of 2,4-DNPH gave maximum intense coloured spot. Similarly, concentration of potassium iodate was varied from 0.2 to 1%. Potassium iodate 0.5% gave best results in terms of colour intensity. Different concentrations of lithium hydroxide solution (0.5-2.5 N) were studied to check its effect on the color density of the green product. 2 N solution of lithium hydroxide served as an ideal for yielding the densest green colored spot. Quantity of methanol used for dissolving diclofenac sodium was also varied from 50 to 100% but it did not show any appreciable change in the final colour intensity.

Effect of Time

To check the effect of time, the colour intensity of the spots was checked by the given procedure after mixing the reagents with diclofenac sodium sample. No appreciable change in the colour intensity of the spots was observed even after 2 hours of mixing the reagents. However, a slight depression in intensity was observed during the 3rd hour. This slight decrease in colour intensity may be attributed to the slow aerial oxidation of the green coloured product.

Calibration curve and statistical data

Under optimum conditions, a calibration graph, shown in Fig. 2, was plotted between the concentration of diclofenac sodium (2-20µg) and the measured optical colour density of the developed spots. The overall density line was selected as a calibration line because of its good correlation coefficient (0.9968) and a direct relationship between concentration and optical colour density. Statistical analysis was run to check the validity of method. The parameters analysed were correlation coefficient, slope, precision, accuracy, the limit of detection, the limit of quantification and relative standard deviation by using ICH harmonized tripartite guidelines. To check the precision and accuracy, 15 samples of the same concentration were analysed, the obtained results showed that the proposed method is accurate and precise and can be applied for analysis of pharmaceutical samples. The results are shown in Table-1.

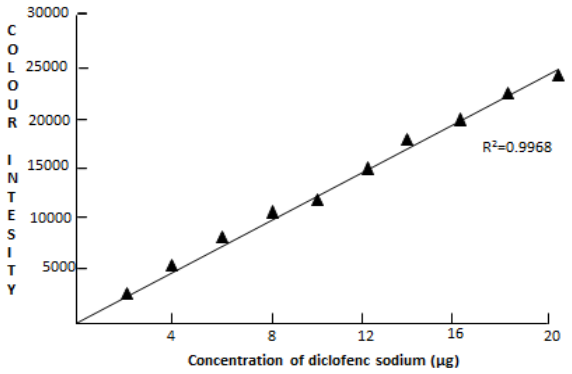

Fig. 2: Calibration Graph by using CSID technique.

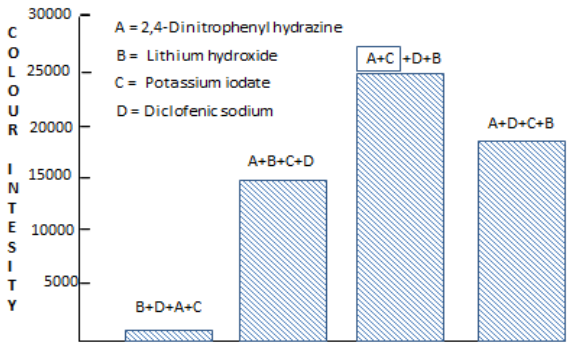

Fig. 3: Effect of order of mixing the reagents.

Table-1: Analytical Parameters and Regression Statics (CSID).

No.	Parameter	Value Obtained
1.	Correlation Coefficient	0.9968
2.	Slope	19.383
3.	Standard Deviation	0.559
4.	Relative Standard Deviation	3.3645
5.	Precision	3.25044
6.	Accuracy	0.83926
7.	LOD (µg/mL)	0.9575
8.	LOQ (µg/mL	2.9017
9.	Confidence Level	1.863
10.	Multiple R	0.998396
11.	R Square	0.996796
12.	Adjusted R Square	0.996395
13.	Standard Error	3.528818
14	Observations	10

Table-2: Assay of Commercial Samples analysed by CSID (*) and Spectrophotometric method (**).

Formulation	Concentration Claimed µg/ml	Concentration Found µg/ml		RSD%		Deviation (%age)	
		(*)	(**)	(*)	(**)	(*)	(**)
Dicloron	30	30.23	30.34	0.159	0.911	0.7	1.2
Voltral	15	15.15	15.83	0.663	0.904	1.0	2.5
Rheumatin	40	40.25	40.96	0.221	0.218	0.57	2.4
Panslay	45	45.21	45.07	0.169	0.121	0.46	1.5
Dicloran Ampoule	25	25.03	25.98	0.080	0.651	0.1	3.92
Voren Ampoule	35	35.41	35.82	0.311	0.123	1.17	2.30

Commercial Samples and Comparison with standard method

To check the practical validity of the new method six commercial samples of diclofenac sodium manufactured by different pharmaceutical companies were obtained from local market and assayed by novel method as well as by a spectrophotometric method [26]. The results obtained in case of both the samples and analytical data are compared in Tables. As revealed by the results and the values of standard deviation found for both methods it can be safely concluded that the proposed method is comparable with reference method in terms of percentage error and relative standard deviation. However, in terms of sensitivity, speed, easy to proceed, quantity of reagents used and need of instrumentation the proposed technique is far better than the spectrophotometric method.

Conclusion

It can be easily concluded from the work presented here that the proposed technique, Computational Image Scanning Densitometry is a simple, fast, sensitive and versatile analytical technique which can be used for the assay of pharmaceutical products. The results of commercial sample obtained by CSID are comparable with those obtained by spectrophotometric method.

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