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SPECTROPHOTOMETRIC DETERMINATION OF DIFLUNISAL FROM ITS FORMULATION BY HYDROTROPY TECHNIQUE

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ABSTRACT

Literature survey reveals that few analytical methods were reported for the estimation of diflunisal by LC method. Hence the present study aims in developing simple, rapid, precise and validated methods for diflunisal in formulations. Organic solvents causes environmental hazards so, to have green synthesis selection of hydrotropic solubilizing agent was preferred. This current investigation is intended to develop a method to determine the assay by U.V. Spectrophotometry, for both bulk of Diflunisal. This method involves direct analysis without any extraction steps, thus it is performed faster, simple and easier. And this method is shown accurate and precise results. By these results this method found to be rapid, simple, accurate, economic method for analysis and quality determination.

Keywords: Diflunisal, UV spectroscopic method, validation studies.

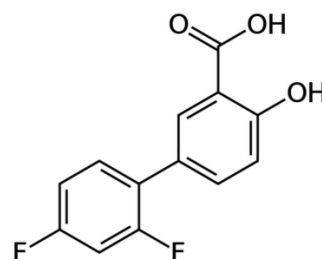
INTRODUCTION

Spectroscopy is a technique that measures the interaction of molecules with electromagnetic radiation. Light in the near-ultraviolet (UV) and visible range of the electromagnetic spectrum has an energy of about 150–400 kJ mol⁻¹. The energy of the light is used to promote electrons from the ground state to an excited state. A spectrum is obtained when the absorption of light is measured as a function of its frequency or wavelength. Molecules with electrons in delocalized aromatic systems often absorb light in the near-UV (150–400 nm) or the visible (400–800 nm) region. Absorption spectroscopy is usually performed with molecules dissolved in a transparent solvent. The absorbance of a solute depends linearly on its concentration and therefore absorption spectroscopy is ideally suited for quantitative measurements. The wavelength of absorption and the strength of absorbance of a molecule depend not only on the chemical nature but also on the molecular environment of its chromophores. Absorption spectroscopy is therefore an excellent technique for following ligand-binding reactions, enzyme catalysis and conformational transitions in proteins and nucleic acids. Spectroscopic measurements

are very sensitive and nondestructive, and require only small amounts of material for analysis.

DRUG PROFILE

Diflunisal



Molecular structure of Diflunisal

Molecular Formula: C₁₃H₈F₂O₃

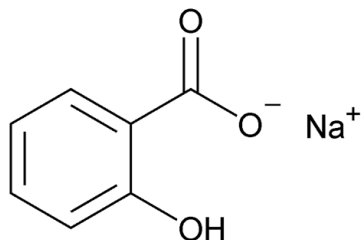
Molecular Weight: 250.198gm/Mole

IUPAC Name: 2',4'-difluoro-4-hydroxybiphenyl-3-carboxylic acid

Diflunisal is a non-steroidal anti-inflammatory drug. Its anti-inflammatory effects are believed to be due to inhibition of both COX-1 and COX-2 which leads to the inhibition of prostaglandin synthesis. Solubility: Insoluble

in water. Soluble in methanol, benzene, chloroform
Dose: 250mg tablet daily (orally).

Hydrotropic Solubilizing Agent Sodium Salicylate



Molecular structure of Sodium salicylate

Molecular Formula: $C_7H_5NaO_3$

Molecular Weight: 160.11gm/mole

IUPAC Name: Sodium 2-hydroxybenzoate

Sodium salicylate is a sodium salt of salicylic acid. It can be prepared from sodium phenolate and carbon dioxide under higher temperature and pressure. Historically, it has been synthesized from methyl salicylate (found in wintergreen plants or the bark of sweet birch tree) by reacting it with an excess of sodium hydroxide and heating it under reflux [1-5].

MATERIALS AND METHODS

Drug Sample

- ❖ The gift sample of pure drug Diflunisal (Bulk powder) was received from MSN Labs, Hyderabad, India.

Chemicals and solvents:

- ❖ Sodium salicylate –Analytical grade
- ❖ Distilled Water-Analytical grade

Instruments used:

- ❖ SHIMADZU UV Pharmspec Spectrophotometer 1700
- ❖ SHIMADZU (ELB 300) Electronic balance
- ❖ SHIMADZU (BL 220H)Electronic balance
- ❖ TOSHIBA (India) Ultra sonicator

Selection of hydrotropic solubilising additive

The hydrotropic solubilising additives used are Urea, Sodium acetate, Sodium benzoate, Sodium salicylate, Sodium salicylate etc. The Urea solution is added to the drug, the drug settles down. The niacinamide solution is added to the drug, the turbidity can be formed. The sodium benzoate solution is added to the drug, the drug settles down. The Sodium salicylate solution is added to the drug, the drug completely dissolved.so Sodium salicylate is used as best hydrotropic solubilising additive.

Selection of wavelength

A wavelength which gives good response for the drug to be detected is to be selected by trial and error

method. From the UV spectra 258 nm was selected as the wavelength for study, fig. 1

Preparation of the stock solution

An accurately weighed 10mg quantity of Diflunisal was transferred into a 10 ml volumetric flask. To this, 4ml of Sodium salicylate solution was added and the flask was shaken for 1 mins to solubilize the drug and the volume was made up to the mark with Distilled water to get a standard stock solution of 1mg/ml. This stock solution used for further dilutions and by using distilled water as solvent for estimation

Preparation of the test solution

Take 100 mg of the Diflunisal drug (sample) and transferred into a 100 mL volumetric flask. To this, 40ml of Sodium salicylate solution was added and the flask was shaken for 5 mins to solubilise the drug and the volume was made up to the mark with Distilled water to get a standard stock solution. By using this solution the concentrations of 2,4,6,8 and 10 $\mu\text{g/ml}$ solutions were prepared for further validation parameters.

Calibration curve preparation

Calibration curve or standard curve is a very important parameter for method validation and assay procedure for the substance. And this factor can be useful to estimate the assay value and drug content present in particular formulation of the drug. This calibration curve linearity can help to detect whether the proposed method is perfect or not.

Calibration curve data were constructed in the range of the expected concentrations of 2 $\mu\text{g/mL}$ to 8 $\mu\text{g/mL}$. Beer's law was obeyed over this concentration range. The regression equation was found to be $y = 0.042x$. The correlation coefficient (r) of the standard curve was found to be 0.990. The stock solutions and working standards were made with water.

By this calibration curve we can analyse the linearity of the method and range of the method [6-12].

VALIDATION OF THE METHOD

The developed method was validated in terms of parameters like precision, linearity and stability studies.

Linearity and Range

Diflunisal was found to be linear in the concentration range of 2- 8 $\mu\text{g/ml}$. The absorbances of these solutions were noted at the selected wavelength, 258 nm. Calibration curve was plotted using concentration Vs absorbance. At a wavelength of 258nm- slope, intercept and correlation coefficient values were found to be 0.026, 0.00333 and 0.992, respectively, fig 2.

Precision

Precision of method was demonstrated by

- Intra day precision

b) Inter day precision

a) Intra day precision

Intra day precision was done by carrying out analysis of standard drug solution at one concentration in the linearity range for three times on the same day and %RSD was calculated, table 4.

b) Inter day precision

Inter day precision was done by carrying out the analysis of standard drug solutions at one concentrations in the linearity range for three days over a period of one week and %RSD was calculated, table 4.

Accuracy

Accuracy is the percentage of analyte recovered by assay from known added amount. Data from nine determinations over three concentration levels covering the specified range were obtained.

LOD (limit of detection)

LOD is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study LOD and LOQ were based on the standard deviation of response and the slope of corresponding curve using following equation:

$$\text{LOD} = 3.3 \sigma / S$$

Where σ is standard deviation of Y-intercept and S is slope of calibration curve. The LOD was found with in limit concentrations (0.3 $\mu\text{g/ml}$ -0.7 $\mu\text{g/ml}$) that 0.0544 $\mu\text{g/ml}$.

LOQ (Limit of quantification)

LOQ is defined as the lowest concentration of calibration curve that can be measured with an acceptable accuracy, precision and variability. The value of LOQ was determined using following equation:

$$\text{LOQ} = 10.3 \sigma / S$$

LOQ (Limit of Quantification) Value is the minimum quantity of drug that can be quantified by the instrument and the value was found to be 0.169 $\mu\text{g/ml}$.

Beers limit

The limits in which beers law obeyed is beers limit. In the UV method development the accuracy, precision the ruggedness, robustness is showed within range called Beers limit. And for Diflunisal, the beers limit range found to be 2 $\mu\text{g/mL}$ to 14 $\mu\text{g/ml}$. Within this range the drug shows accuracy, linearity, precision, ruggedness, robustness

Molar Absorptivity

This is the important factor for determining the absorptive property of a drug in 1 mole concentration. And this value can be useful in determining the absorbance of drug in molar concentrations. This for identifying the shifts of the maximum absorbance of the drug during the method development. The molar absorptivity of the Diflunisal was found to be $\times 10^4 \text{ L mole}^{-1} \text{ cm}^{-1}$ [13-24].

RESULTS

For the UV spectroscopic conditions were optimized to get best correlation coefficient and LOD, LOQ. The optimum wavelength for detection and quantification was 258 nm.

Fig. 1. Standard curve of Diflunisal

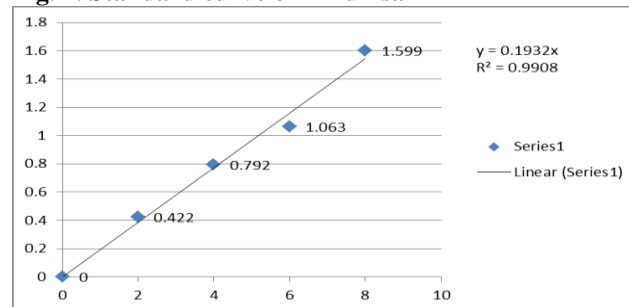


Table 1. standard curve values

| Concentration($\mu\text{g/mL}$) | Absorbance |
|-----------------------------------|------------|
| 0 | 0 |
| 2 | 0.422 |
| 4 | 0.792 |
| 6 | 1.063 |
| 8 | 1.599 |

Table 2. Method Validation Parameters of Dexibuprofen

| Parameters | Results |
|--------------------------------------|---|
| λ_{max} | 258 nm |
| Beer's law limit | 2-8 $\mu\text{g/ml}$ |
| Molar absorptivity | $2.657 \times 10^4 \text{ L mole}^{-1} \text{ cm}^{-1}$ |
| Regression equation ($Y = mx + c$) | $y = 0.042 + 0.00253x$ |
| Slope (m) | 0.042 |
| Intercept (c) | 0.00253 |
| Correlation coefficient (r) | 0.992 |
| Relative standard deviation (%) | 0.072 |
| LOD Value | 0.0501 |
| LOQ Value | 0.1565 |

Table 3. Diflunisal intra-day and inter-day precision

| Sample ($\mu\text{g/ml}$) | Intra-day precision | | Inter-day precision | |
|-----------------------------|---------------------|-------|---------------------|-------|
| | Absorbance | RSD | Absorbance | RSD |
| 6 | 1.376 | 0.099 | 1.104 | 0.072 |
| | 1.675 | | 0.947 | |
| 8 | 1.063 | 0.086 | 1.114 | 0.075 |
| | 1.122 | | 0.965 | |

There was no interference from the diluents, excipients present in the pharmaceutical formulation. To check the Linearity, standard calibration curve of the drug was constructed by plotting using absorbance vs. concentration of standard solution and the curve showed good linearity over a concentration range of 2-8 $\mu\text{g/ml}$. The regression equation of the drug was found by plotting absorbance (y) vs. Concentration(x) $\mu\text{g/ml}$. The LOD and LOQ values of Diflunisal were found to be 0.5019 $\mu\text{g/ml}$ and 1.5656 $\mu\text{g/ml}$ respectively. The Precision of the method was determined by repeatability (intra day) and intermediate precision (inter day). Precision was expressed as the RSD of the results. The value obtained for the precision studies indicates good repeatability and low inter day variability.

DISCUSSION AND CONCLUSION

Diflunisal is a NSAID. It acts by inhibition of cyclo-oxygenase, which is involved in prostaglandin synthesis. Hydrotropy refers to the ability of a concentrated solution of a chemical compound to increase the aqueous solubility of another compound [usually a sparingly soluble organic compound]. Compounds that have this property are called 'hydrotopes'. Sodium benzoate, sodium salicylate, sodium acetate, sodium ascorbate, niacinamide and sodium citrate are the most popular examples of hydrotropic agents which have been used to solubilise a large number of poorly water-soluble compounds. Hydrotropic solution, trisodium citrate was employed as solubilizing agent.

Diflunisal is soluble in organic solvents. But it causes the environmental hazards. To have green synthesis that Trisodium citrate used as hydrotropic solubilising agent. At the same time it is non-UV absorbable.

In this method the hydrotropic solubilising agent as Tri sodium citrate is used. A wavelength of 258 nm was selected for study. The developed method was validated as per ICH guidelines. Calibration graphs were plotted absorbance Vs concentration of standard drug solutions. The slope, intercept and correlation coefficient values were found to be 0.042, 0.00253, 0.992. The results show that within the concentration range tested, there was excellent correlation between absorbance and concentration. Diflunisal was found to be linear in the range of 2 to 8 $\mu\text{g/ml}$. The LOD of Diflunisal was found to be 0.0501 $\mu\text{g/ml}$. The LOQ of Diflunisal was found to be 0.1565 $\mu\text{g/ml}$. Precision of the developed method was studied under intraday precision; inter day precision. Low % RSD values show that the developed method is precise.

This current investigation is intended to develop a method to determine the assay by U.V. Spectrophotometry, for both the bulk of Diflunisal. This method involves direct analysis without any extraction steps, thus it is performed faster, simple and easier. And this method is shown accurate and precise results. By these results this method found to be rapid, simple, accurate, economic method for analysis and quality determination.

REFERENCES

1. Anonymous. www.R_x list
2. Anonymous. www.wikipedia.com
3. Maheshwari RK, Chaturvedi SC, Jain NK. Titrimetric Analysis of Aceclofenac in Tablets Using Hydrotropic Solubilization Technique. *Indian Drugs*, 43, 2006, 516-8.
4. Maheshwari RK, Chaturvedi SC, Jain NK. Application of Hydrotropic Solubilization Phenomenon in Spectrophotometric Analysis. *Indian Drugs*, 42, 2005, 760-3.
5. Jain NK, Agrawal RK, Singhai AK. Formulation of aqueous injection of carbamazepine. *Pharmazie*, 45, 1990, 221-4.
6. Jain NK, Patel VV. Hydrotropic Solubilization. *Eastern Pharmacist*, 29, 1986, 51-4.
7. Beckett & Stenlake. Practical pharmaceutical chemistry, Fourth Edition, CBS Publishers and Distributors, 296.
8. Sethi PD. Quantitative Analysis of Drugs in Pharmaceutical Formulations, Third Edition, CBS Publishers and Distributors, 1997.
9. Spectroscopy Donald L. Pavia, Gary M. Lampman, George S. Kriz, James R. Vijaan Rifino CB. Pharmaceutical Process Validation, Switzerland, Edition 2, 2003, 1-43.
10. International Conference on Harmonization (ICH), Validation of Analytical Procedures: Text and Methodology Q2 (R1), November 2005.

11. The United States Pharmacopoeia, Rockville, MD, Edition 3, 2003, 2320.
12. Srikant Nayak, Rashmi Ranjan Sarangi, Susanta Kumar Panda, Arun Kumar Dash, Sangram Kumar Rath, Satyanarayana Rath. UV- spectrophotometric method for simultaneous Estimation of paracetamol and ondancetron in bulk and their formulation. *International Journal of Biological & Pharmaceutical Research*, 2(2), 2011, 45-49.
13. Satyanarayana Rath, Susanta Kumar Panda, Rashmi Ranjan Sarangi, Arun Kumar dash, Sangram Kumar Rath, Srikant Nayak. UV-spectrophotometric method for simultaneous Estimation of metoprolol and amlodipine in bulk and Their formulation. *International Journal of Biological & Pharmaceutical Research*, 2(2), 2011, 50-54.
14. Sangram Kumar Rath, Rashmi Ranjan Sarangi, Susanta Kumar Panda, Arun Kumar Dash, Satyanarayana Rath, Srikant Nayak. UV- spectrophotometric method for simultaneous Estimation of drotaverine hydrochloride and Aceclofenac in bulk and their formulation. *International Journal of Biological & Pharmaceutical Research*, 2(2), 2011, 55-59.
15. Görög S. Ultraviolet-Visible Spectrophotometry in Pharmaceutical Analysis. CRC Press, New York, 1994.
16. Kumar CHA, Kumar TA, Gurupadayya BM, Sloka SN, Reddy BMR. Novel spectrophotometric determination of valacyclovir and cefotaxime using 1, 2-napthaquinone-4-sulfonic acid sodium in bulk and pharmaceutical dosage form. *Arch. Appl. Sci. Res*, 2, 2010, 278-287.
17. Mahmoud AM, Khalil NY, Darwish IA, Aboul-Fadl Y. Selective spectrophotometric and spectrofluorometric methods for the determination of amantadine hydrochloride in capsules and plasma via derivatization with 1,2-naphthoquinone-4-sulphonate. *Int. J. Anal. Chem*, 2009, 8.
18. Padmarajaiah N, Kumar HRA, Vasantha RA, Yathirajan HS. Novel reagents for the sensitive spectrophotometric determination of flutamide, an anticancer drug in pharmaceutical preparations. *Int. J. Pharm*, 235, 2002, 113-120.
19. Al-Momani IF. Spectrophotometric determination of selected cephalosporins in drug formulations using flow injection analysis. *J. Pharm. Biomed. Anal*, 25, 2001, 751-757.
20. Pesez M, Bartos J. Colorimetric and fluorimetric analysis of organic compounds and drugs. Marcel Dekker Inc., New York, 1974, pp. 628-630.
21. Robinson RA, Stokes RH. Electrolyte solutions, the measurement and interpretation of conductance, chemical potential, and diffusion in solutions of simple electrolytes. 2nded., London, Butterworths, 1968.
22. Lindbohm ML, Taskinen HT, Sallman M, Hemminki K. Spontaneous abortions among women exposed to organic solvents. *Am J Indust Med*, 17, 2007, 449-463.
23. Wennborg H, Bonde JP, Stenbeck M, Olsen J. Adverse reproduction outcomes among employee in biomed-ical research laboratories. *Scand. J. Work Environ. Health*, 28, 2002, 5-11.
24. Wennborg H, Lennart B, Harri V, Gösta A. Pregnancy outcome of personnel in swedish biomedical re-search laboratories. *J. Occup. Environ. Med*, 42, 2000, 438-446.