Spectrophotometric Estimation of Ethamsylate and Mefenamic Acid from a Binary Mixture by Dual Wavelength and Simultaneous Equation Methods

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Goyal, et al.: Simultaneous Analysis of Ethamsylate and Mefenamic Acid

Two simple, accurate, economical and reproducible spectrophotometric methods for simultaneous estimation of two-component drug mixture of ethamsylate and mefenamic acid in combined tablet dosage form have been developed. The first developed method involves formation and solving of simultaneous equation using 287.6 nm and 313.2 nm as two wavelengths. Second developed method is based on two wavelength calculation. Two wavelengths selected for estimation of ethamsylate were 274.4 nm and 301.2 nm while that for mefenamic acid were 304.8 nm and 320.4 nm. Both the developed methods obey Beer's law in the concentration ranges employed for the respective methods. The results of analysis were validated statistically and by recovery studies.

Key words: Ethamsylate, mefenamic acid, simultaneous analysis, two wavelength calculation method, simultaneous equation method

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Ethamsylate (ESLT) is diethylammonium-2,5-dihydroxybenzenesulphonate and is used as a haemostatic agent for prevention and treatment of capillary hemorrhage associated with haemostasis, menorrhagia and post-partum haemorrhage. The drug is official in British Pharmacopoeia with estimation of drug by potentiometric method. Literature survey reveals that one spectrophotometric and one LC methods are reported for the estimation of ESLT from pharmaceutical formulations.

Mefenamic acid (MFNC) is 2-[(2,3-dimethylphenyl) amino]benzoic acid and is used as an analgesic and antiinflammatory agent. The drug is official in British Pharmacopoeia with estimation of the drug by non-aqueous titrimetric method. Literature survey reveals that one spectrophotometric, one HPLC and three LC methods have been reported for the estimation of MFNC from pharmaceutical formulations. However none of the methods is yet reported for simultaneous estimation of two drugs from combined pharmaceutical dosage forms.

Developed spectrophotometric methods were found to be simple, rapid, accurate, reproducible and economical in comparison to routine extractive or colorimetric methods used for analysis of single drug and have been used successfully for determination of two components from combined tablet dosage form.

A PC based Systronic, UV/Vis double beam spectrophotometer (model No. 2101) with spectral bandwidth of 2 nm and wavelength accuracy ±0.5 nm (with automatic wavelength correction) and wavelength readability 0.1 nm increment was employed for all measurements using a matched pair of 10 mm quartz cells.

Standard bulk drug samples of ESLT and MFNC were provided by Ochoa Laboratories Pvt. Ltd., New Delhi. Methanol was used as solvent for the preparation of stock solution and for further dilutions. The tablet samples of combined dosage form of ESLT and MFNC [Sylate-M250 (Emcure Pharmaceuticals Ltd., Pune), Sylate-M500 (Emcure Pharmaceuticals Ltd., Pune) and Eklot-MF (Kontest Pharmaceuticals Ltd., Mumbai)] were procured from the local pharmacy.

In the first method, pure drug sample of ESLT and MFNC were dissolved separately in methanol so as to give several dilutions of standard in concentration range of 0-50 μg/ml of each drug. All dilutions were scanned in wavelength range of 200.0 450.0 nm. Two wavelengths selected for formation and solving of simultaneous equation were 287.6 nm and 313.2 nm. Absorptivity coefficient of both the drugs was determined at selected wavelengths. Absorptivity coefficient for ESLT at 287.6 nm and 313.2 nm were 37.40 cm⁻¹g⁻¹ l and 157.78 cm⁻¹g⁻¹ l while respective values for MFNC were 328.80 cm⁻¹g⁻¹ l and 111.62 cm⁻¹g⁻¹ l. Set of two simultaneous equation thus framed were, \( A_1 = 157.78 C_1 + 111.62 C_2 - I \) and \( A_2 = 37.40 C_1 + 328.80 C_2 - II \), where \( A_1 \) and \( A_2 \) are absorbance of sample solution at 287.6 nm and 313.2 nm, respectively. \( C_1 \) and \( C_2 \) are concentration of ESLT and MFNC, respectively in sample solution in g/l. Validity of above framed equation was checked by preparing five mixed standards using pure sample of two drugs, measuring their absorbance at respective wavelengths and calculating concentration of two components. The result of validation studies was found satisfactory.

Twenty tablets were accurately weighed and average weight per tablet was determined. Tablets were grounded to fine powder and tablet powder equivalent to 100 mg ESLT was weighed and extracted four times with 20 ml portions of methanol and filtered through Whatman filter paper no. 41 into a 100 ml volumetric flask. Washed residue with methanol and added washings to filtrate, volume of filtrate was made to 100 ml mark with methanol. From above filtrate 10 ml was diluted to 100 ml with methanol and finally 1 ml was further diluted to 10 ml with methanol. Absorbance of this final dilution was measured at 287.6 nm and 313.2 nm, respectively, and concentration of two drugs in the sample was calculated using above framed simultaneous equations-I and II. Results of analysis of tablet formulation are reported in Table 1.

For method II, set of two wavelengths \( \lambda_1 \) (274.4 nm) and \( \lambda_2 \) (301.2 nm) for estimation of ESLT and \( \lambda_3 \) (304.8 nm) and \( \lambda_4 \) (320.4 nm) for estimation of MFNC were selected on basis of the principle that absorbance difference between two points on a mixture spectra is directly proportional to concentration of component of interest and independent of interfering component. Five mixed standards of pure drugs containing different concentration of ESLT and MFNC were prepared in methanol. All standards were scanned at
respective set of selected wavelengths. Absorbance difference was measured and respective calibration curve was plotted.

Tablet samples were prepared in a similar manner as for method I. Final dilution was analyzed by scanning at respective set of wavelength and absorbance difference values were noted and concentration of ESLT and MFNC was calculated from the respective calibration curve. Results of analysis are reported in Table 1.

To study the accuracy, reproducibility and precision for both the developed methods recovery studies were carried out by the addition of standard drug solution to pre-analyzed tablet sample with proper dilutions at three different concentration levels with in the range of linearity for both the drugs. Results of recovery studies were found to be satisfactory and are reported in Table 1. The proposed methods for simultaneous estimation of ESLT and MFNC in combined tablet dosage form were found to be simple, accurate, rapid and economical. The values of recovery were close to 100% indicating reproducibility of the methods.

First developed method involving formation and solving of simultaneous equation based on accurate determination of absorptivity coefficient of two drugs at two selected wavelengths. Once the equation is framed then it is just required to measure the absorbance of sample solution at selected wavelengths and few calculations that can be manually done. Framed equations were validated using laboratory prepared mixed standards of two drugs which gave satisfactory results.

Second developed method for simultaneous analysis of ESLT and MFNC from combined dosage form make use of two wavelength calculation so as to remove interference between two components. Proper selection of two wavelengths for estimation of a component is critical.

The results of analysis of two drugs from tablet formulation using both the developed methods were found close to 100 percent for both ESLT and MFNC, standard deviation was satisfactorily low indicating accuracy and reproducibility of the methods. Recovery studies were satisfactory which shows that there is no interference of excipients. The developed methods were found to be simple, rapid, accurate and can be used for routine estimation of two drugs from tablet formulations.

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| Method   | Batch | Label claim mg/Tab. | % Label claim estimated* | Standard deviation | % Recovery** |
|----------|-------|----------------------|--------------------------|--------------------|-------------|
|          |       | ESLT     | MFNC     | ESLT     | MFNC     | ESLT     | MFNC     | ESLT     | MFNC     | ESLT     | MFNC     |
| I        | A     | 500      | 500      | 100.43   | 101.10   | 0.6066   | 0.2586   | 100.54   | 100.78   |
|          | B     | 250      | 250      | 100.74   | 100.96   | 0.3524   | 0.3601   | 101.10   | 100.83   |
|          | C     | 250      | 250      | 100.36   | 100.90   | 0.5788   | 0.1289   | 101.22   | 101.00   |
| II       | A     | 500      | 500      | 99.81    | 100.38   | 0.9200   | 0.9585   | 100.18   | 99.86    |
|          | B     | 250      | 250      | 100.73   | 101.34   | 0.9170   | 0.9600   | 100.40   | 101.63   |
|          | C     | 250      | 250      | 101.64   | 99.42    | 0.9150   | 0.9433   | 101.86   | 99.82    |

A is Sylate-M500 (Emcure Pharmaceuticals Ltd., Pune), B is Sylate-M250 (Emcure Pharmaceuticals Ltd., Pune) and C is Eklot-MF (Konext Pharmaceuticals Ltd., Mumbai). ESLT is ethamsylate; MFNC is mefenamic acid. *Each value is an average of five estimations; **Average of recovery studies at three different concentration levels. Method I is simultaneous equation method; Method II is two wavelength calculation method.
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Ravi, et al.: Colon targeted drug delivery system

A novel colon targeted tablet formulation was developed using pectin as carrier and diltiazem HCl and indomethacin as model drugs. The tablets were coated with inulin followed by shellac and were evaluated for average weight, hardness and coat thickness.

In vitro release studies for prepared tablets were carried out for 2 h in pH 1.2 HCl buffer, 3 h in pH 7.4 phosphate buffer and 6 h in simulated colonic fluid. The drug release from the coated systems was monitored using UV/Vis spectroscopy.

In vitro studies revealed that the tablets coated with inulin and shellac have limited the drug release in stomach and small intestinal environment and released maximum amount of drug in the colonic environment. The study revealed that polysaccharides as carriers and inulin and shellac as a coating material can be used effectively for colon targeting of both water soluble and insoluble drugs.

Key words: Colon targeting, natural polymers, pectin, inulin and shellac

In recent times, colon targeted drug delivery systems have gained importance for the systemic delivery of protein and peptide drugs. This is because the peptide and protein drugs get destroyed or inactivated in acidic environment of the stomach or by pancreatic enzymes in the small intestine.

Drug targeting to colon is also useful when a delay in drug absorption is desired from therapeutic point of view, such as treatment of diseases that have peak symptoms in the early morning like nocturnal asthma, angina or arthritis. Among the different approaches to achieve colon specific drug delivery, the use of polymers, specifically biodegraded by colonic bacterial enzymes holds promise.

The important bacteria present in the colon such as Bacteroides, Bifidobacterium, Eubacterium, Peptococcus, Lactobacillus, Clostridium secrete a wide range of reductive and hydrolytic enzymes such as β-glucuronidase, β-xylosidase, β-galactosidase, α-arabinosidase, nitroreductase, azoreductase, deaminase and urea hydroxylase. These enzymes are responsible for degradation of di-, tri- and polysaccharides.

Pectin is a polysaccharide obtained from plant cell walls. Inulin is a polysaccharide, which is obtained from plants such as onion, garlic, chicory and artichoke. Being soluble in water, pectin is not able to shield its drug load effectively during its passage through the stomach and small intestine. Hence a coat of a considerable thickness is required to protect the drug core in simulated in vivo conditions.

The aim of the present study was to develop pectin based matrix tablet with sufficient mechanical strength and promising in vitro mouth-to-colon release profile. The matrix tablets were coated with inulin and shellac to target the tablets to colon. The coated tablets were evaluated for weight gain, coat thickness and in vitro dissolution studies.

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