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Novel spectrophotometric determination of Valacyclovir and Cefotaxime using 1, 2-napthaquinone-4-sulfonic acid sodium in bulk and pharmaceutical dosage form

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ABSTRACT

A new, simple and sensitive spectrophotometric method for the determination of valacyclovir and cefotaxime has been developed. The method is based on the condensation of valacyclovir and cefotaxime with 1, 2- napthaquinone-4- sulfonic acid sodium (NQS) in alkaline media to yield orange colored products respectively. Valacyclovir and cefotaxime showed maximum absorbance at 495nm and 475nm with linearity was observed in the concentration range of 20-120 µg/ml and 20-140 µg/ml respectively. The relative standard deviations of 0.363% for valacyclovir and 0.66% for cefotaxime were obtained. The recoveries of valacyclovir and cefotaxime injections are in the range 96.01±0.52 and 98.12±0.96 respectively. The proposed method is simple, rapid, precise and convenient for the assay of valacyclovir and cefotaxime in commercial injection preparations.

Key words: Valacyclovir, Cefotaxime, condensation, 1, 2- napthaquinone 4- sulfonic acid sodium, spectrophotometry, pharmaceutical formulation.

INTRODUCTION

Valacyclovir Chemically *L*-valine-2-[(2-amino-1, 6-dihydro-6-oxo-9-hipurin-9-yl) methoxy] ethyl ester is the *L*-valyl ester prodrug of the antiviral drug acyclovir that exhibits activity against herpes simplex virus types, 1 (HSV-1) and 2 (HSV-2) and vericellazoster virus [1]. The mechanism of action of acyclovir involves the highly selective inhibition of herpes virus DNA replication, via enhanced uptake in herpes virus-infected cells and phosphorylation by viral thymidine kinase. The substrate specificity of acyclovir triphosphate for viral, rather than cellular, DNA polymerase contributes to the specificity of the drug [2, 3]. Valacyclovir is available as tablet dosage form in the market. Few HPLC methods were reported for the determination of valacyclovir in pharmaceutical formulations [4, 5], in biological fluids [6-10]

and spectrophotometric methods were reported [11, 12]. There are some few stability indicating HPLC methods were also developed for valacyclovir in pharmaceutical dosage forms [13-15].

Cefotaxime chemically (6R, 7R)-3-[(acetyloxy) methyl]-7-[[(2Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate is official in Indian Pharmacopoeia[16]. It is a third generation cephalosporin, a broad antibacterial spectrum and is resistant to β-lactamases. Several analytical methods have been reported. High-performance liquid chromatography (HPLC) [17-19], thin-layer chromatography [20], and spectrophotometric [21–23] techniques were reported.

1, 2-naphthoquinone-4-sulphonic sulphonate (NQS) has been used as a chromogenic reagent for the spectrophotometric determination of many pharmaceutical amines [24, 25]. However, the reaction between NQS with valacyclovir and cefotaxime has not been investigated so far. The present study describes the evaluation of NQS as a chromogenic reagent in the development of simple and rapid spectrophotometric method for the determination of valacyclovir and cefotaxime in its pharmaceutical dosage forms.

MATERILAS AND METHODS

Apparatus

A Shimadzu UV-visible spectrophotometer model 1800 with 1 cm matched quartz cell was used for the absorbance measurements. Systonics electronic balance was used for weighing the samples.

Reagents

All employed chemicals were of analytical grade and high-purified water was used throughout the study. Cefotaxime pure sample was obtained as a gift sample from Biochem Pharmaceutical Industries Limited, Mumbai, India. Valacyclovir pure sample obtained as a gift sample from Hetero Drugs limited, Hyderabad, India.

1, 2-Naphthoquinone-4-sulphonate (NQS) 0.5 %(w/v)

0.5 g of NQS was accurately weighed transferred into a 100 ml calibrated flask, dissolved in 10ml distilled water, and make up the volume up to the mark with distilled water to obtain a solution of 0.5% (w/v). The solution was freshly prepared and protected from light during the use.

10×10^{-3}N (0.01N) Sodium hydroxide solution

0.2 g of sodium hydroxide is accurately weighed and transferred into a 500.0ml volumetric flask and made up to the mark with distilled water.

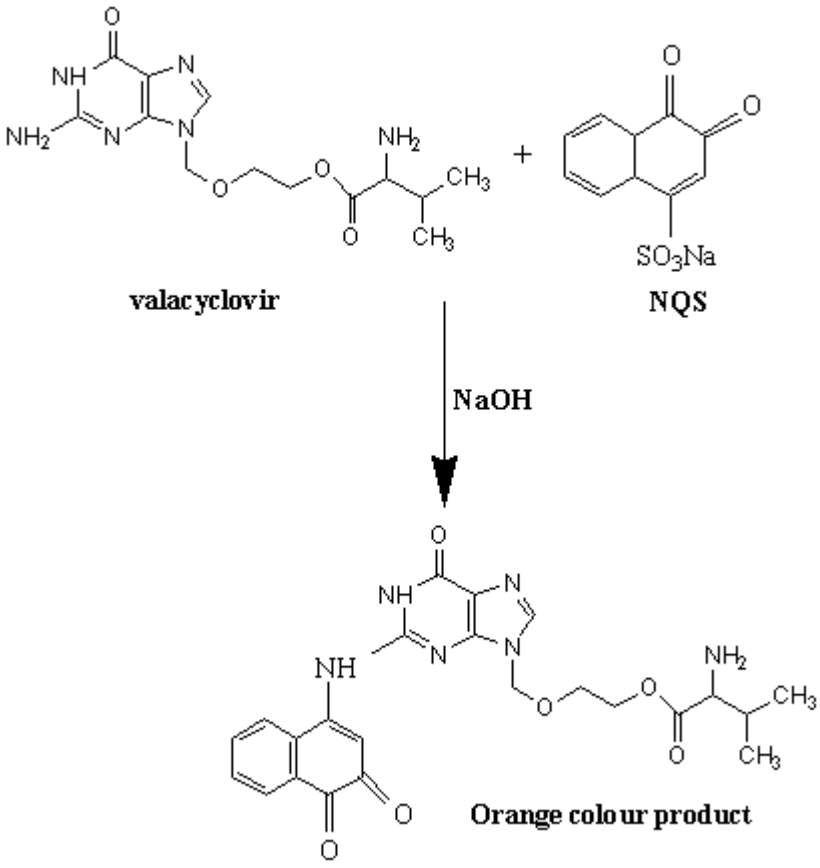
Standard solutions

Valacyclovir and cefotaxime stock solutions (1000 µg/ml) were prepared separately in distilled water. Working solutions of the drug were prepared by dilution of the stock solution. The tablet form of valacyclovir which are used in the determination was Valcivir with labelled amount of 500mg and manufactured by Cipla Limited, Mumbai India. The injection form of cefotaxime which are used in the determination was Biotax® with a labelled amount of 1 g and manufactured by Biochem Pharmaceutical Industries Limited, Mumbai, India.

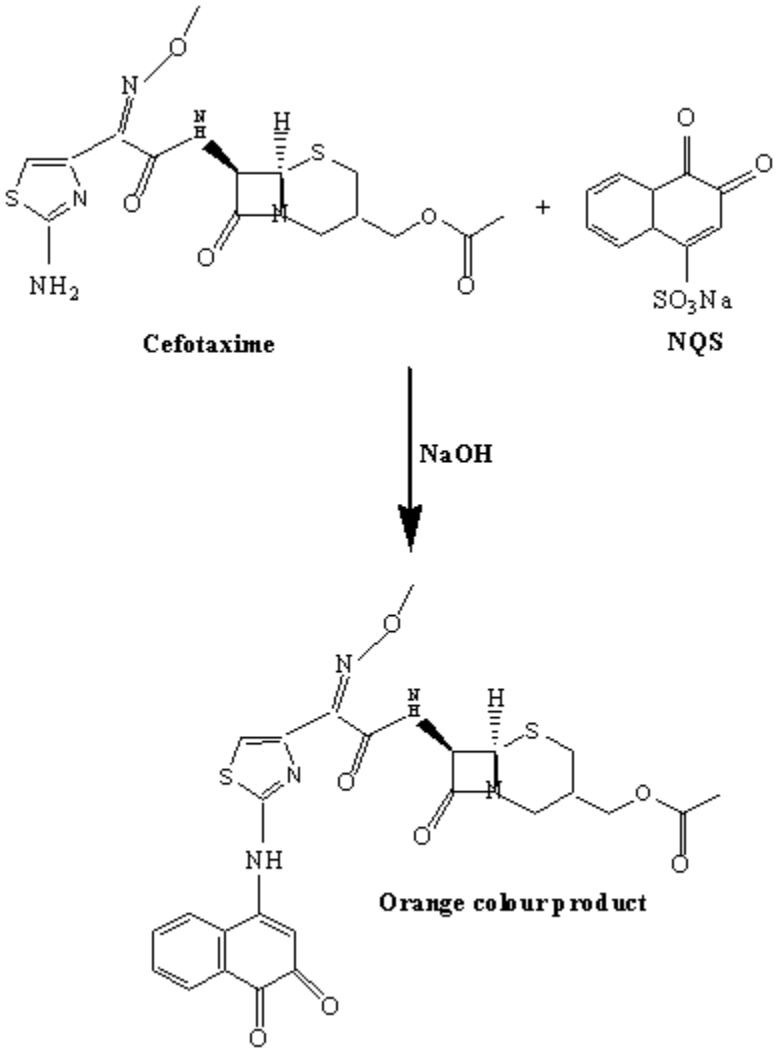
Selection of Analytical Wavelengths for valacyclovir and cefotaxime

A 1 ml quantity of 0.5% NQS solution and 1ml of $10 \times 10^{-3}N$ sodium hydroxide were added into two test tubes and 0.5 ml of valacyclovir and cefotaxime stock solutions were added. The immediate orange coloured complex was formed (scheme 1 & 2). The solutions were made up to 10ml with water. The absorption spectrums of the complex were determined against blank solution and the wavelengths of maximum absorption (λ_{max}) of the products of the reactions were noted.

Scheme 1: Mechanism of reaction of valacyclovir with NQS and formation orange colored product



Scheme 2: Mechanism of reaction of cefotaxime with NQS and formation orange colored product



Effect of Reagent Concentration

The effect of varying the concentration of NQS was carried out using reagent concentrations of 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5% in 10 x 10^{-3}N NaOH. After mixing 1ml of each reagent concentration with the drug solutions of valacyclovir and cefotaxime and made up to 10 ml with water, the absorbance readings were recorded at 495nm and 475nm respectively on the UV-visible spectrophotometer.

Optimization Studies

Effect of NQS Concentration

The studying of NQS concentrations revealed that the reaction was dependent on NQS reagent. The absorbance of the reaction solution increased as the NQS concentration increased, and the highest absorption intensity was attained at NQS concentration of 0.25 % (w/v). Higher NQS concentrations up to 1.25 % had no effect on the absorption values. Further experiments were carried out using 0.5 %.

Effect of Alkalinity and pH

To generate the nucleophiles from valacyclovir/cefotaxime and also to activate the nucleophilic substitution reactions, alkaline medium was necessary. Different inorganic bases were tested: sodium hydroxide, disodium hydrogen phosphate, and sodium bicarbonate, all prepared as

aqueous solution of a concentration range of 0.5 – 30 x 10^{-3} N. Best results were obtained in case of sodium hydroxide where with other bases either precipitation of white colloid occurred upon diluting the reaction solution with organic solvent, high blank readings, non reproducible results, and/or weak sensitivity were observed. Studies for optimization of sodium hydroxide concentration revealed that the optimum concentration was 10 x 10^{-3}N (Fig 1).

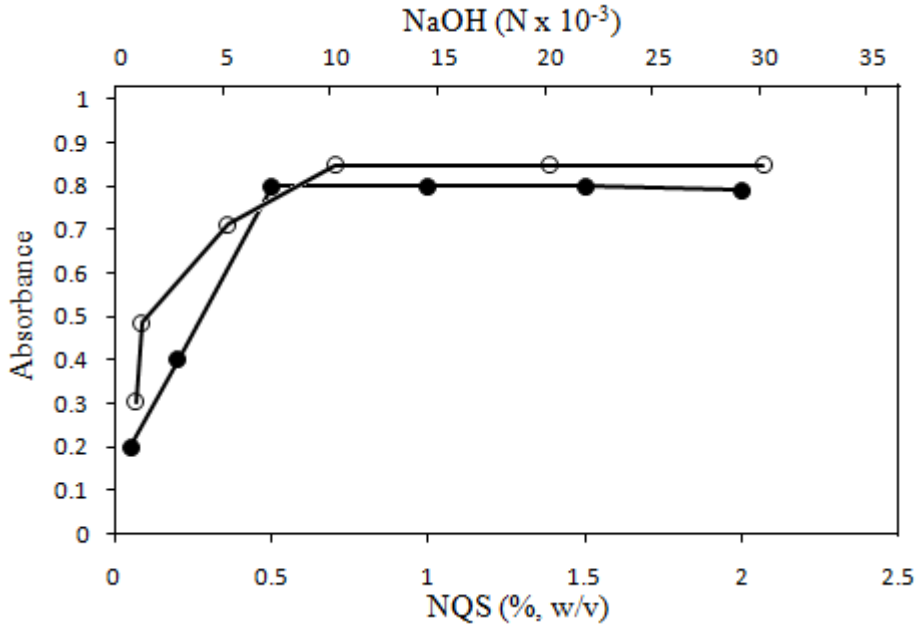


Fig 1: Effect of pH on the reaction of valacyclovir/cefotaxime with NQS.

In a separate series of experiments, the influence of pH on the absorbance of valacyclovir/cefotaxime-NQS product was investigated. The results revealed that the absorbances at pH < 6 were close to 0, indicating that under acidity, valacyclovir/cefotaxime have difficulty to react with NQS (Fig 2).

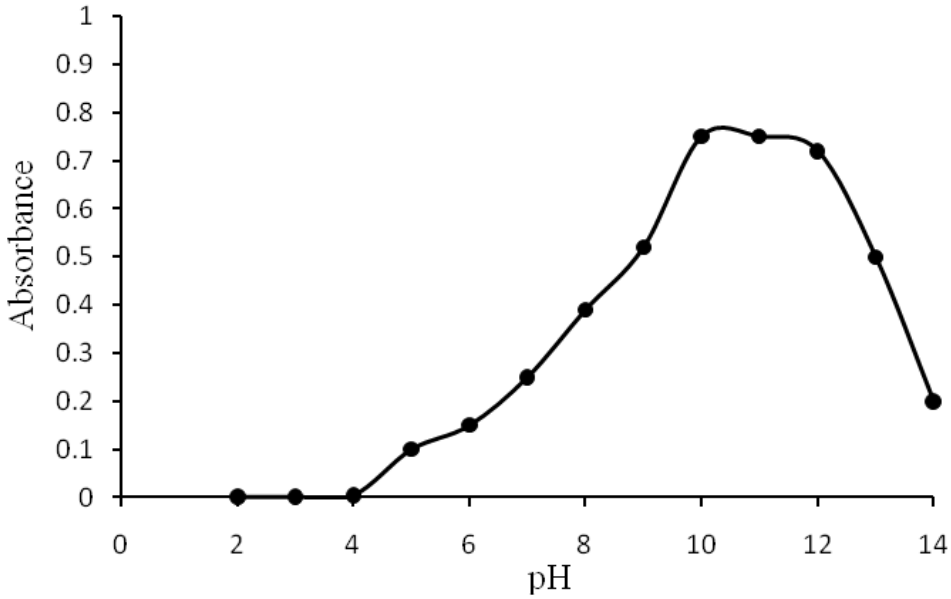


Fig 2: Effect of pH on the reaction of valacyclovir and cefotaxime with NQS

At pH > 6, the absorbance increased rapidly with the increase in the pH, as the amino group of valacyclovir and cefotaxime turns into the free-NH, facilitating the nucleophilic substitution reaction. The maximum absorption values were attained in the range of pH at 10 –11.5. At pH > 11.5, the absorbance of solution obviously decreased. This was attributed probably to the increase in the amount of hydroxide ion that holds back the condensation reaction between valacyclovir/cefotaxime and NQS. In order to keep the high sensibility for determination of valacyclovir and cefotaxime, the experiment was carried out at pH 11.5.

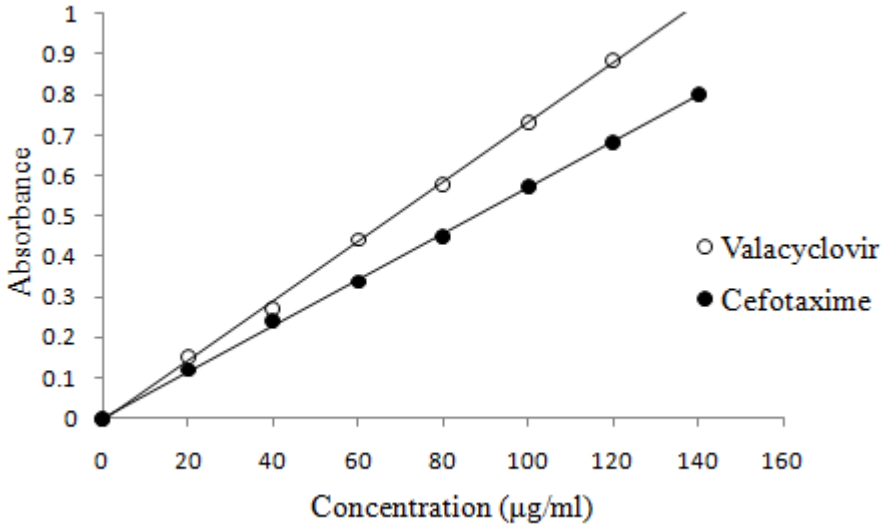


Fig 3: Calibration graphs of valacyclovir and cefotaxime, conc. (valacyclovir) 20-120 µg/ml; conc. (cefotaxime) = 20-140 µg/ml

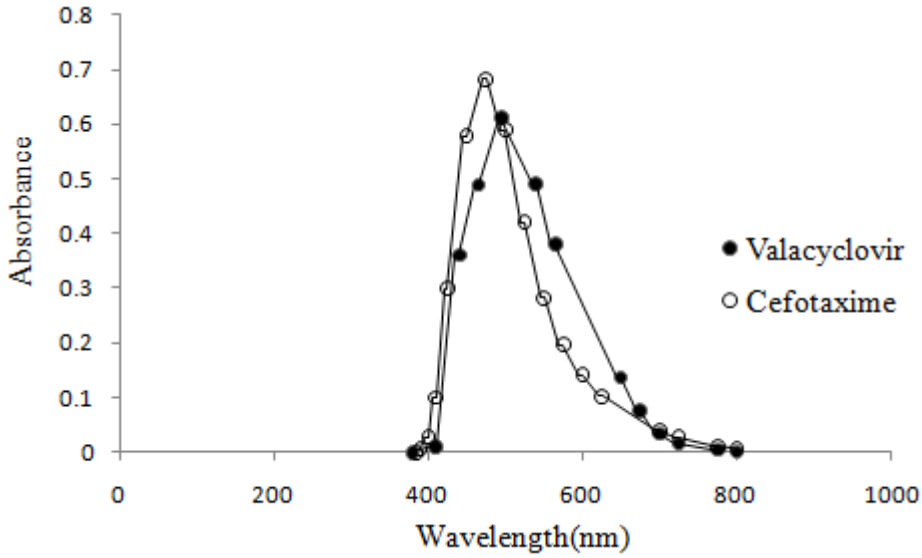


Fig 4: Absorption spectra of NQS with valacyclovir and cefotaxime against the reagent blank

Preparation of calibration curve

Standard solutions of valacyclovir and cefotaxime in water, having final concentrations in the range of 20-120 µg/ml and 20-140 µg/ml respectively, were transferred into a series of 10 ml volumetric flasks, to these solutions 1 ml of 10 x 10^{-3}N sodium hydroxide is added, 1 ml of 0.5% NQS is added. The mixture was then gently shaken until the appearance of orange color. The

contents were diluted up to 10 ml with distilled water. The absorbance of each solution was measured at 495nm and 475nm respectively against the reagent blank prepared in the same manner, without the analyte and the calibration curve and absorption spectra are represented in the (Fig 3 and 4) respectively.

Analysis of commercial pharmaceutical preparations

Tablet

Twenty tablets were weighed and their contents are mixed thoroughly. An accurately weighed portion of powder equivalent to the labelled strength (100 mg) of valacyclovir was weighed into a 100 ml volumetric flask containing about 75 ml of distilled water. It was shaken thoroughly for about 5-10 min, filter thoroughly with whatman filter paper to remove insoluble matter and diluted to the mark with distilled water to prepare 1000 µg/ml solution. An aliquot of this solution was diluted with water to obtain a concentration of 40 µg/ml. Then to that solution 1 ml of 0.01N sodium hydroxide is added, 1 ml of 0.5% NQS is added. The mixture was then gently shaken until the appearance of orange colour. The contents were diluted up to 10 ml with distilled water.

Injection

An appropriate amount of cefotaxime was dissolved in water for injection so as to prepare 1000 µg/ml solution. An aliquot of this solution was diluted with water to obtain concentration of 40 µg/ml. To that solution 1 ml of 10 x 10^{-3}N sodium hydroxide is added, 1 ml of 0.5% NQS is added. The mixture was then gently shaken until the appearance of orange colour. The contents were diluted up to 10 ml with distilled water.

General procedure:

Several standard solutions of valacyclovir and cefotaxime were taken in individual standard flasks. To each standard flask, 1 ml of 10 x 10^{-3}N sodium hydroxide and 1 ml of 0.5% NQS was added. The mixtures were then shaken until the appearance of orange colour. The absorbance was measured at λ max at 495 nm and 475 nm for valacyclovir and cefotaxime respectively against a blank similarly prepared by omitting the drug solution with water. The concentration of valacyclovir and cefotaxime in each standard flask was obtained by interpolating the corresponding absorbance value from Beer's plot of standard valacyclovir and cefotaxime solutions.

Quantification

The limits of the Beer's law, the molar absorptivity and the Sandell's sensitivity values were evaluated. Regression analyses of the Beer's law plots at their respective λmax values revealed a good correlation. Graphs of absorbance versus concentration showed zero intercept, and are described by the regression equation, $Y = bX + c$ (where Y is the absorbance of a 1 cm layer, b is the slope, c is the intercept and X is the concentration of the drug in µg/ml) obtained by the least-squares method. The results are summarized in Table 1.

Validation

Six tubes containing varying volumes of valacyclovir and cefotaxime stock solution, (0.2-1.2 and 0.2-1.4 ml) with respective concentrations, (20-120 µg/ml and 20-140 µg/ml) were prepared. 1.0 ml of 0.5% w/v NQS was added to each of these tubes. Then 1.0 ml of 0.05N sodium hydroxide was also added. Then the volume is made up to the volume. The absorbance readings of each of the mixtures of both the drug mixtures were then recorded at 495 and 475nm respectively. These processes were repeated three times and on each occasion fresh stock solutions of valacyclovir

and cefotaxime were prepared and used. The average absorbance reading was obtained from the determinations, and used to generate the calibration curves. Linear regression analysis was used to calculate the slope, intercept and coefficient of determination (R^2) of each calibration line. The limit of detection (LOD) was computed from the calibration graphs using the equation 3.3 σ/s where σ is the standard deviation of three blank determinations and s is the slope of the calibration curve. The limit of quantisation (LOQ) was calculated as 10 σ/s.

Table 1. Optical Characteristics and Statistical Data for the Regression Equation of the Proposed Method

Parameter	Values	
	Valacyclovir	Cefotaxime
λ_{max}/ nm	495 nm	475 nm
Beer's law limits (µg/ml)	20-120	20-140
Molar absorptivity (1 /mol/cm)	0.179×10^4	0.271×10^4
Correlation coefficient (R)	0.9991	0.9996
Sandell's sensitivity(ng cm^{-2})	0.1325	0.166
Regression equation (y)	y = 0.0074x - 0.0046	y = 0.0057x + 0.0033
Slope, *b*	0.0074	0.0057
Intercept, *c*	0.0046	0.0033
Relative standard deviation%	0.363	0.66
Recovery	96.01±0.52	98.12±0.96
Repeatability	0.363	0.66
Limit of detection (µg/ml)	0.445	0.914
Limit of quantification (µg/ml)%	1.33	2.77

Y = bX + c, where X is the concentration of drug in µg/ml; Average of six determinations

Precision: The precision of the method was determined by replicate analysis of five separate solutions of five separate solutions of the working standards at two concentration levels of each drug. Relative standard deviations are also calculated which indicates good precision of the proposed methods.

Table 2. Analysis of injections, recovery and ruggedness of the assay of valacyclovir and cefotaxime by proposed

Sample	Drug Present(mg)	Found* ±SD; % and their comparison with official method
		NQS method
Valacyclovir tablet Valcivir	500	96.01±0.52
Intraday analysis	500	96.01.±0.52
Interday analysis	500	95.53±0.65
Cefotaxime injection Biotax®	1000	98.12±0.96
Intraday analysis	1000	98.12±0.96
Interday analysis	1000	97.99±0 .82

Robustness and ruggedness: Robustness was examined by evaluating the influence of a small variation of the method variables including the concentration of analytical reagent and the pH of the sodium chloride solution. It was found that small variations in these variables did not affect the method significantly. This was an indication of the reliability of the proposed method during its routine application for the investigated drugs. The ruggedness was tested by applying the proposed method of analysis for both the drugs using the same operational conditions. Results

obtained from inter-day RSD and within-day RSD variations were found to be reproducible and are represented in the Table 2.

RESULTS AND DISCUSSION

Derivatisation of valacyclovir and cefotaxime were attempted in the present study for the development of spectrophotometric method for its determination. The present method is based on the reaction between the NQS and valacyclovir and cefotaxime molecules. The NQS reagent reacts with valacyclovir and cefotaxime at the free NH_2 group. The reagent blank has negligible absorbance in the range used for detection of the valacyclovir and cefotaxime. Beer's law is obeyed in the range of 20-120 µg/ml for valacyclovir and 20-140 µg/ml for cefpirome.

Derivatisation using NQS has attracted considerable attention for quantitative analysis of many pharmaceutically active compounds. In the present investigation, NQS forms a coloured complex with valacyclovir and cefotaxime in alkaline conditions and their absorbances were measured at 495nm and 475nm respectively. Because of the presence of amine as chromophoric group in valacyclovir and cefotaxime molecules, derivatization of these compounds was attempted in the present study for the development of spectrophotometric methods for its determination. NQS has been used as chromogenic and fluorogenic reagent for primary and secondary amines, however, its reaction with valacyclovir and cefotaxime has not been investigated yet. Therefore, the present study was devoted to explore NQS as a derivitising reagent in the development of spectrophotometric method for the determination of valacyclovir and cefotaxime in pharmaceutical dosage forms.

Optimisation of the spectrophotometric conditions was intended to take into account the various goals of method development. Analytical conditions were optimised via a number of preliminary experiments. The effect of NQS concentration was studied and found that 0.5% gave good absorbance values so further experiments were carried out using 0.5 % NQS. To generate the nucleophiles from valacyclovir and cefotaxime activate the nucleophilic substitution reactions, alkaline medium was necessary. Different inorganic bases were tested: sodium hydroxide, disodium hydrogen phosphate, and sodium bicarbonate, all prepared as aqueous solution of a concentration range of 0. 01 - 0.05 N. Best results were obtained in case of sodium hydroxide in the concentration of 1ml of 0.01 N solution.

Stability of the Chromogen:
Under the optimum conditions, the reaction between valacyclovir/cefotaxime and NQS was completed within 2 minutes at room temperature, and the absorbance no longer changed after standing for up to 40 minutes. The effect of time on the stability of the chromogen was studied by following the absorption intensity of the reaction solution (after dilution) at different time intervals. It was found that the absorbance of the chromogen remains stable for at least 4 hours. This allowed the processing of large batches of samples and their comfortable measurements with convenience. This increased the convenience of the methods as well as made it applicable for large number of samples.

CONCLUSION

The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Moreover, the methods are free from interference by common additives and excipients. The wide applicability

of the new procedures for routine quality control was well established by the assay of valacyclovir and cefotaxime in pure form and in pharmaceutical preparations.

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