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Assay of Nelfinavir Mesylate in Pharmaceutical Formulations by Visible Spectrophotometry

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Three simple and sensitive visible spectrophotometric methods (A-C) for the determination of nelfinavir mesylate (NEL) in bulk and pharmaceutical formulations are described. Methods A and B are based on the formation of ion-association complexes between NEL and wool-fast blue BL (WFB BL, λ_{max} 590 nm) or Tropaeolen 000 (TP 000, λ_{max} 480 nm). Method C is based on the formation of diazo coupling product of NEL with diazotized *p*-nitroaniline (DPNA, λ_{max} 480 nm). Regression analysis of Beer's law plots showed good concentration ranges (0.5–5.0, 1.9–20 and 2.0–25) μg/mL for methods A, B and C respectively.

Key Words: Extractive, Spectrophotometric determinations, Nelfinavir mesylate.

INTRODUCTION

Nelfinavir mesylate (NEL) is an antiviral drug and is chemically known as 3-isoquinolinecarboxamide, [3S-[2(2S*,3S*),3α,4aβ,8aβ]]-N-(1,1-dimethyl-ethyl) decahydro-2-[2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl) amino]-4-(phenylthio)butyl]monomethane sulfonate. It is an inhibitor of human immuno deficiency virus (HIV) protease. This inhibition prevents the cleavage of viral polyproteins, resulting in the formation of immature non-infection viral particles.

A number of methods such as HPLC[1–8], MS[9] and IR[10] were reported for the estimation of NEL. No visible spectrophotometric method has been reported for the assay of NEL in literature. The authors have made some attempts in developing visible spectrophotometric methods and succeeded in developing three methods based on the formation of ion-association complex with WFB BL (method A), TP 000 (method B) or diazo coupling product of NEL with diazotized *p*-nitroaniline (method C). These methods are successfully extended to pharmaceutical formulations containing nelfinavir mesylate.

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EXPERIMENTAL

Instrumentation: A Milton Roy Spectronics 1201 and a Systronics 106 digital spectrophotometer with 1 cm matched quartz cells were used for the spectral and absorbance measurements. An Elico LI-120 digital pH-meter was used for pH measurements.

Reagents: All the chemicals and reagents were of analytical grade and the solutions were prepared in triply distilled water.

Aqueous solutions of WFB BL (Fluka, 3.26×10^{-3} M), TP 000 (Fluka, 5.70×10^{-3} M), glycine-HCl buffer solution of pH 1.5 (method A) and 0.1 M hydrochloric acid (method B) and PNA (Koch Light Labs, 7.25×10^{-3} M), $NaNO_2$ (S.D. Fine Chemicals, 1.45×10^{-2} M) and NaOH (S.D. Fine Chemicals, 1 M) were prepared for method C.

Preparation of standard drug solution

A 1 mg/mL solution was prepared by dissolving 100 mg of pure NEL in 100 mL of 0.01 M HCl and this stock solution was diluted stepwise with 0.01 M HCl to obtain the working standard solutions of concentrations 20 μg/mL for method A, 80 μg/mL for method B and 100 μg/mL for method C.

For pharmaceutical formulations

The tablet powder equivalent to 100 mg of NEL was accurately weighed and dissolved in chloroform and filtered. For methods A, B and C, the filtrate was evaporated to dryness and the residue was dissolved in 100 mL of 0.01 M HCl to achieve a concentration of 1 mg/mL, from which suitable dilutions were performed for methods A, B and C as mentioned above.

Recommended Procedures

Methods A and B: Into a series of 125 mL separating funnels containing aliquots of standard NEL solution [0.5–2.5 mL, 20 μg mL^{-1} (method A), 0.5 to 2.5 mL, 80 μg mL^{-1} (method B)], 6.0 mL of buffer solution pH 1.5 (method A), or 0.1 M HCl (method B) and 2.0 mL of dye solution WFB BL (method A), or TP 000 (method B) were added. The total volume of aqueous phase in each separating funnel was adjusted to 15 mL with distilled water and 10 mL of $CHCl_3$ was added. The contents were shaken for 2 min. The two phases were allowed to separate and the absorbances of the separated organic layers were measured at 590 nm (method A) or 480 nm (method B) against a similar reagent blank similarly prepared within the stability period (1–60 min) at laboratory temperature (28 ± 5°C). The amounts of NEL in methods A and B were computed from their respective calibration curves.

Method C: Into a series of 10 mL graduated test tubes 1.0 mL of PNA solution and 1.0 mL of $NaNO_2$ solution were successively added and allowed to stand for 2 min. Later, aliquots of the standard NEL solution (0.5–2.5 mL, 100 μg mL^{-1}) were delivered into the test tubes. Then I.5 mL of NaOH solution was added and the volume in each tube was made up to 10 mL with distilled water. The absorbances were measured at 480 nm against a reagent blank. The amount of NEL in a sample was computed from the calibration curve.

RESULTS AND DISCUSSION

Conditions under which the reaction of NEL with each dye and diazocoupling fulfills the essential analytical requirements were investigated. All the experimental conditions studied were optimized at room temperature (28 ± 5°C) and were established by varying one parameter at a time and observing the effect on the absorbance of the coloured species.

In view of developing methods of analysis suitable for assaying small quantities of NEL, we tried six acidic dyes, namely, WFB BL, TP 000, TP 00, NB 12BR, AG or ARS as colour producing agents at various pH ranges, by a dye salt partition technique. Among these dyes, wool fast blue BL (WFB BL, method A) and tropaeolin 000 (TP 000, method B) were selected as being the most suitable with high λ_{max} and ε_{max} values in the formation of ion-association complexes which are quantitatively extracted into chloroform. These dyes also gave low absorbance for the reagent blank. Chloroform was suggested as a solvent of choice for the extraction of coloured complex with respect to maximum stability.

In order to establish the optimum pH range (for method A) or acid strength (for method B), the NEL was allowed to react with the respective dye in aqueous solution buffered between pH 1.0–6.0 (method A) or solution in dil. HCl ranging from 0.05-1.5 M (method B) and the complex formed was extracted into chloroform for absorbance measurement. The results show that a quantitative extraction was produced with a pH 1.1–1.5 (method A) or an acid strength of 0.08-0.12 M HCl (method B). All subsequent studies were carried out with pH 1.5 (for method A) or with 0.1 M HCl (for method B). The pH was adjusted using glycine-HCl buffer solution (this buffer was chosen on account of its elevated complexing ability, which could be of use in overcoming interferences). The volume of this buffer added (4–10 mL) had no effect in method A. A 6.0 mL portion of 0.1 M HCl solution was found to be optimal in method B. The maximum shaking time was determined by varying the shaking time from 1–10 min, although 1 min was sufficient, prolonged shaking has no adverse effect on the extraction and 2 min was selected for this study. A ratio of 1 : 1.5 (methods A and B) of organic to aqueous phases were required for efficient extraction of the coloured species and lowest reagent blank reading. It was found that better reproducibility and a lowest reagent blank was achieved if the dye was purified by extraction with chloroform initially.

In order to establish optimum conditions for method C, the effect of various parameters such as volumes of PNA, $NaNO_2$, NaOH, waiting time for diazotization and for maximum colour formation and the stability of coloured species were studied at room temperature. Optimum volumes 0.8–1.2 mL, each of PNA and $NaNO_2$ were found to be adequate to produce diazonium chloride. 10 min time was found to be necessary for diazotization. So 1 mL each of PNA and $NaNO_2$ were preferred for further investigations. Minimum amount of 1.0 mL of NaOH was found to be suitable to maintain alkaline conditions in the method for the coupling of NEL. The final colour (λ_{max}, 480 nm) in method C was attained within 5 min and remained stable for 30 min. The same results were obtained over the

temperature range 10–30°C. So it is not necessary to cool the solution in ice. However, low absorbances were observed at temperatures beyond 35°C.

Analytical data

The optical characteristics such as λ_{max}, Beer's law units, molar extinction coefficient, Sandell's sensitivity, regression equation and correlation coefficient obtained by linear least square treatment[11] of the results for the systems involving NEL with the mentioned dyes or DPNA are presented in Table-1. The precision of each method was tested by estimating six replicates of NEL within Beer's law limits. The per cent standard deviation and the per cent range of error at 95% confidence limit are given in Table-1.

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION, ACCURACY OF THE METHODS PROPOSED IN THE DETERMINATION OF NEL

Parameters	Method A	Method B	Method C
λ_{max} (nm)	590	480	480
Beer's Law limits (μg mL^{-1})	0.5–5.0	1.9–20	2.0–25
Molar absorptivity (L mol^{-1} cm^{-1})	7.16×10^4	1.77×10^4	1.69×10^4
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	0.009	0.037	0.038
Regression equation $y = a + bc$*:			
Slope (b)	0.107	0.026	0.026
Intercept (a)	0.0022	0.0009	–0.0004
Correlation coefficient (r)	0.9999	0.9999	0.9998
Relative Standard Deviation (%)†	0.575	0.724	0.489
% of range error (confidence limit):			
(i) 0.05 level	0.604	0.760	0.513
(ii) 0.01 level	0.947	1.190	0.805
% error in bulk sample‡	0.308	0.311	–0.261

*Y = a = bC, where C is concentration.
†Average of six determinations considered.
‡Average of three determinations.

In order to confirm the utility of the proposed methods, they were applied to the estimation of NEL in tablets and the results are presented in Table-2. The results obtained by the proposed and UV reference methods for the formulations were compared statistically[12] by means of F- and t-tests and were found not to differ significantly. As an additional check of accuracy of proposed methods, recovery experiments were performed by adding a fixed amount of the NEL to the pre-analyzed formulation and the results are also summarized in Table-2. The per cent recoveries were found to be within ±1%. The results indicate that the proposed procedures do not differ significantly from reference method.

Chemistry of coloured species

In methods A and B, the formation of the coloured complex is based on the basic nature of the drug (NEL), which under specified experimental conditions forms ion

TABLE-2
DETERMINATION OF NEL IN PHARMACEUTICAL FORMULATIONS

Samples[a]	Labelled amount (mg)	Amount found by proposed methods[c]			Reference method[b]	Recovery by proposed methods[d]		
		Method A	Method B	Method C		Method A	Method B	Method C
Tablet I	250	250.03 ± 0.59 F = 2.17 t = 0.10	250.10 ± 0.71 F = 1.23 t = 0.25	249.01 ± 0.82 F = 2.07 t = 0.43	250.09 ± 0.69	100.01 ± 0.83	100.04 ± 0.59	99.60 ± 0.33
Tablet II	250	250.05 ± 0.69 F = 1.34 t = 0.051	247.93 ± 0.39 F = 1.75 t = 1.21	249.13 ± 0.99 F = 1.44 t = 0.27	249.63 ± 1.77	100.02 ± 0.71	99.17 ± 0.26	99.65 ± 0.39
Tablet III	250	250.06 ± 0.42 F = 2.13 t = 0.64	250.92 ± 0.34 F = 1.03 t = 1.66	249.90 ± 0.52 F = 2.53 t = 0.94	250.45 ± 1.24	100.02 ± 0.72	100.37 ± 0.68	99.96 ± 0.21
Tablet IV	250	249.81 ± 0.36 F = 1.90 t = 0.39	250.06 ± 0.25 F = 1.70 t = 1.65	250.08 ± 0.52 F = 1.54 t = 1.17	249.26 ± 1.59	99.92 ± 0.22	100.02 ± 0.55	100.03 ± 0.21

[a] Four different batches of tablets from a pharmaceutical company.

[b] Developed in the laboratory using methanol solvent (λ_{max}, 253 nm).

[c] Average ± standard deviation of six determinations; the t- and F- values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, t = 2.57, F = 5.05.

[d] After adding three different amounts of the pure labelled to the pharmaceutical formulation, each value is an average of three determinations.

association complexes with certain acidic dyes (WFB BL or TP 000) which are extractable into chloroform. The stoichiometric ratio of NEL to WFB BL or TP 000 was determined with the slope ratio method[13] and found to be 1 : 1. The quantitative measure of the effect of complexation on acid-base equilibrium is most likely to be interpretable in terms of electronic, steric and other effects of complexing. The drug NEL (1 mole) and the oppositely charged form of the dye (1 mole) behave as a single unit, being held together by electrostatic attraction.

In method C, the diazo coupling reaction may be considered as a proton eliminating condensation of a diazonium salt with another compound possessing an active hydrogen atom. Coupling of a diazonium salt formed from aromatic primary amine (*p*-nitroaniline)[14] takes place in mild acid conditions. Substitution usually proceeds to para position to the active group or to ortho if para is substituted. This method involves two steps. In the first step *p*-nitroaniline is treated with nitrous acid (from sodium nitrite and HCl) to get diazotized *p*-nitroaniline (DPNA). In the second step the drug is added to DPNA to get diazo coupled product.

Conclusions

The proposed methods are applicable for the assay of drug (NEL) and have the advantage of wider range under Beer's law limits. The decreasing order of sensitivity and λ_{max} among the proposed methods are A > B > C and A > B = C respectively. The proposed methods are simple, selective and can be used in the routine determination of NEL in bulk samples and formulations with reasonable precision and accuracy.

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