Pergamon

0039-9140(94)00198-7

Talanta, Vol. 41, No. 12, pp. 2131–2136, 1994

0039-9140/94 $7.00 + 0.00

SOLID SURFACE ROOM TEMPERATURE PHOSPHORIMETRY ANALYSIS OF RESERPINE IN PHARMACEUTICAL FORMULATIONS

R. Q. AUCÉLIO and A. D. CAMPIGLIA*

Departamento de Quimica, Universidade de Brasilia, Brasilia, DF, Brazil, CEP 70910-900

(*Received* 16 *December* 1993. *Revised* 10 *June* 1994. *Accepted* 20 *June* 1994)

Summary—A simple, rapid and sensitive method for reserpine analysis has been developed based on solid surface room temperature phosphorimetry. Phosphorescence emission was induced by the reserpine hydrolysis reaction in basic medium. Chromatography paper previously treated for background reduction was employed as a solid substrate. Four heavy atom salts and sodium dodecyl sulfate were tested for maximum signal intensity. A calibration curve with a linear dynamic range of three orders of magnitude (10^{-7}–$10^{-4}M$) was obtained. A 1.9 ng limit of detection was estimated and recoveries of 98.7 and 100.3% were obtained in two dosage forms with different pharmaceutical matrices.

Reserpine (methyl 1,2-didehydro-2,7-dihydro-11,17α-dimethoxy-3β,20α-yohimban-16β-carboxylase, 18β-trimethoxy benzoate ester) has been the subject of intensive research on account of its therapeutic properties.[1] Mainly extracted from *Rauwolfia serpentina* roots, this alkaloid is employed in pharmaceutical formulations as an anti-hypertensive, tranquilizer and sedative.[2]

The large variety of dosage forms has caused the development of numerous assay procedures for reserpine determination. These include spectrophotometric,[3,4] polarographic,[5] radioimmunoassay,[6] HPLC[7–9] and fluorimetric methods.[10–12] Most fluorimetric assays take advantage of reserpine oxidation to induce fluorescence emission. The oxidation can be achieved by chemical reaction,[10] but the procedures are usually time-consuming, taking from 30 to 90 min to reach maximum fluorescence intensity. Catalysis[11] and ultraviolet radiation[12] have been recently proposed to speed up the reaction process and reduce analysis time.

We now report a new quantitative method based on solid surface room temperature phosphorimetry (SSRTP).[13,14] Phosphorescence emission from reserpine was directly induced by its dissolution in basic medium. A nanogram limit of detection was estimated by using a low background paper substrate[15] previously treated with appropriate phosphorescence enhancers.[16,17] Satisfactory recoveries were obtained for two pharmaceutical formulations with different dosage forms.

*Author to whom correspondence should be addressed.

EXPERIMENTAL

Instrumentation

The temperature effect on the hydrolysis reaction of reserpine was studied employing magnetic stirrer-hot plates (Corning, U.S.A.). Continuous stirring was accomplished using teflon magnetic stir bars.

For background reduction, the paper substrate was irradiated in a Rayonet photochemical reactor (The Southern N.E. Ultraviolet Co., Middletown, CT, U.S.A.) using five lamps with maximum wavelengths (λ_{max}) of emission at 254 nm and seven with λ_{max} at 300 nm.

An Aminco-Bowman spectrophotofluorimeter (SLM Instruments, Urbana, IL, U.S.A.) equipped with an Aminco-Keirs rotating-can phosphoroscope attachment was used to collect all RTP spectra and intensity measurements. The spectrofluorimeter was equipped with a 150 W xenon arc lamp (Conrad-Hanovia, Newark, NJ, U.S.A.) as excitation source, and a potted IP21 photomultiplier (Hamamatsu Corp., Middlesex, NJ, U.S.A.) with an S4 spectral response as a detector. An SLM-Aminco microphotometer was used to amplify

the signal. The amplified signal was fed to an X–Y recorder (Model 7010B, Hewlett-Packard, Palo Alto, CA, U.S.A.). A laboratory-constructed sample holder was used for all measurements.[18]

The nitrogen flow employed throughout all measurements was previously passed by a silica gel bed to remove possible water vapor traces.

Reagents

Whatman No. 1 chromatography paper was used as a solid substrate. Tridistilled (in glass) water and analytical-reagent grade chemicals were used throughout. Ethanol, acetone and silver nitrate were purchased from Merck (Brazil). Reserpine, caffeine, furosemide, ascorbic acid and hydrochlorotriazide were obtained from Sigma (U.S.A.). Sodium dodecyl sulfate (specially purified for biochemical work) and lead (II) nitrate were from BDH Chemicals Ltd (U.K.). Thallium (I) nitrate was acquired from Fluka (Switzerland) and sodium hydroxide from Vetec (Brazil). All reagents were employed without further purification.

Pharmaceutical preparations

Two pharmaceutical formulations commercially available in our country were employed to test the recovery of our method. Gross[R] preparations consisted of tablets containing reserpine as the only active ingredient (0.25 mg per unit). Each tablet of Adelfan-Esidrex[R], a Ciba-Geigy formulation, contained 0.1 mg reserpine, 10 mg dihydralazine acid sulfate and 10 mg hydrochlorotriazide.

Procedures

Paper background reduction. The treatment for paper background reduction has been previously described.[15] Strips of chromatography paper were water extracted in a Soxhlet apparatus for 8 hr, introduced into quartz tubes and exposed to UV radiation for the same period of time. The strips were then removed and cut into rectangular pieces (10 × 17 nm) to fit in the sample holder.[18]

Reserpine hydrolysis reaction. Reserpine hydrolysis reaction was performed in basic medium by adding a proper volume of a 2*M* NaOH solution to volumetric flask containing reserpine dissolved in ethanol/water or acetone/water. The final NaOH concentration was 0.4*M*.

Temperature effect. The temperature effect on the hydrolysis reaction of reserpine was studied by heating the alkaline solutions under continuous stirring. Solvent evaporation was minimized by covering the solution beakers with watch glasses. Most of the condensed vapor was returned to reserpine solutions by washing the covers with the organic phase of the solvent system employed.

Standard and sample preparation. Standard solutions for the calibration curves were prepared in acetone–aqueous 0.4*M* NaOH (30:70 v/v). For the analysis of commercial preparations containing hydrochlorotriazide, 100 mg of this compound were added to all standard solutions.

The analysis of commercial preparations was performed by mixing three tablets containing the equivalent of 0.3 mg reserpine with a pestle and a mortar. The resultant powder was dissolved in acetone–aqueous 0.4*M* NaOH (30:70 v/v). After vacuum filtering, the solution was brought to a final volume of 50 ml.

Measurement of RTP emission. By means of a microliter pipette, 5 μl of analyte solution were deposited on the surface of the paper substrates. In some cases, the paper had been previously spotted with 5 μl of heavy atom solution or with 5 μl of surfactant followed by 5 μl of heavy atom solution. The spotted substrates were vacuum-dried at room temperature for approximately 1 hr and placed in a desiccator with silica until measurement time. The drying time was later reduced by placing the samples under an IR lamp for 10 min. Possible light effects were prevented by covering the desiccator with aluminum foil. Prior to measurements, a flow of dry nitrogen was directed to the surface of the paper substrate for 5 min. All RTP intensities and spectra were recorded under a continuous flow of dry nitrogen to avoid possible quenching effects from oxygen and moisture.[13,14]

RESULTS AND DISCUSSION

Preliminary attempts

SSRTP determination of reserpine has been previously reported by Winefordner and co-workers.[19,20] Nanogram levels of detection were estimated using filter paper discs pretreated with both 1*M* NaI and 1*M* NaOH. According to the authors,[19] the absence of

NaI or NaOH in the matrix of the compound resulted in no phosphorescence emission.

Preliminary attempts carried out in our laboratory confirmed the necessity of basic hydrolysis prior to phosphorimetric detection. No analyte signal was observed from reserpine solutions (ethanol–water 50:50 v/v) spotted on low background paper substrates. Previous treatment of solid supports with heavy atom (HA) salts [1*M* NaI, 0.5*M* $Pb(NO_3)_2$, 0.1*M* $TlNO_3$ and 0.5*M* $AgNO_3$][16] resulted in no phosphorescence signal, even in the presence of a 2% aqueous solution of sodium dodecyl sulfate (NaDS), a surfactant which usually increases the HA efficiency.[17,21-23]

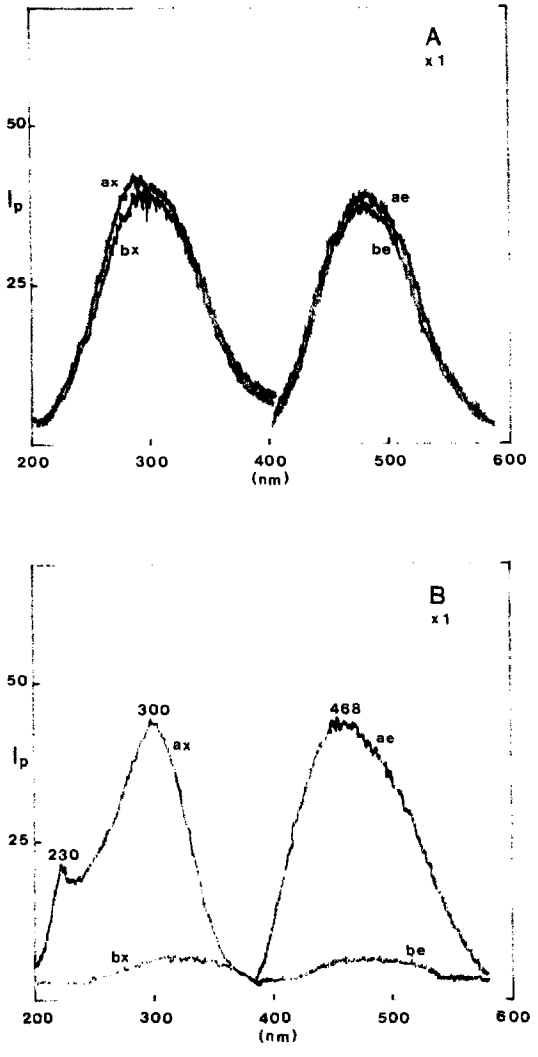

Fig. 1. Excitation (a_x) and emission (a_e) phosphorescent spectra of reserpine ($10^{-4}M$) in ethanol–aqueous 0.4*M* NaOH (50:50 v/v) using Whatman #1 chromatography paper, without treatment (A) and after treatment (B). b_x and b_e refer, respectively, to excitation and emission phosphorescence spectra of the blank.

Table 1. Temperature effect on the phosphorescence emission of reserpine basic solutions*

Temperature (°C)†	25	40	60	70	80
I_A/I_B‡	5.7	7.5	8.3	9.2	8.7

*$10^{-4}M$ solutions prepared in ethanol–water (50:50 v/v).
†Heating time = 60 min.
‡I_A = phosphorescence intensity of analyte, I_B = phosphorescence intensity of blank. Six determinations of analyte signals and respective blanks were employed to calculate I_A/I_B. All measurements were performed at the excitation (300 nm) and emission (468 nm) maximum wavelengths of analyte. The precision observed was within 10–15%.

RTP emission of reserpine basic hydrolysis product

A significant improvement of the analyte signal was observed from reserpine solutions prepared in ethanol–aqueous 0.4*M* NaOH (50:50 v/v). Contrary to previously reported data,[19] no HA salt was required to detect phosphorescence emission from basic solutions. The analyte signal was observed as a consequence of the low background signal of the paper substrate (see Fig. 1).

While performing these studies, we noticed that the phosphorescence emission from a 24-hr-old basic solution was higher than the one detected from a fresh basic solution. Attributing the fact to the slow kinetics of reserpine basic hydrolysis, we monitored the signal intensity—in 24 hr intervals—during a period of 3 days. The highest analyte-to-background signal ratio (I_A/I_B) was observed after 48 hr of solution preparation. During this period of time, there was an enhancement of approximately 2.3 times in the I_A/I_B ratio.

As a tentative means of speeding up the hydrolysis process, and considering the fact that 0.4*M* NaOH was the highest possible NaOH concentration in ethanol–water (50:50 v/v), we studied the effect of temperature and heating time on the RTP signal of reserpine basic solutions. Table 1 shows the I_A/I_B ratios obtained from solutions heated for 60 min at several temperatures. Although there was a remarkable increase in the intensity of the analyte signal, the maximum excitation (λ_{exc}) and emission (λ_{em}) wavelengths remained constant for all solutions. Apparently, the temperatures tested did not affect the nature of the phosphorescent compound resultant from the basic hydrolysis of reserpine. On the other hand, the velocity of the reaction process was substantially favored, which is an attractive asset for a routine analysis method.

Since the highest I_A/I_B ratio was obtained at 70°C, we studied the effect of heating time of this temperature. After heating a solution for 30 min, the value of the I_A/I_B ratio was statistically similar to the one obtained after 60 min of heating time [for the statistical test, a probability (P) of 0.05 and six determinations ($N_1 = N_2 = 6$) for each ratio were considered]. The ratio remained constant after a period of 120 min, showing that the maximum value was reached with only 30 min of heating. Although it was faster than 60 min, a half an hour heating step was still inconvenient from a routine analysis point of view.

Aware of the fact that the nature of the solvent can modify the rate of reactions, we tested acetone as the organic phase in reserpine basic solutions. Analyte solutions were prepared in acetone–aqueous 0.4M NaOH (30:70 v/v) and their RTP emissions were compared to those obtained from heated ethanol–aqueous 0.4M NaOH (50:50 v/v) solutions. The I_A/I_B ratios measured from fresh acetone solutions were statistically similar ($P = 0.05$; $N_1 = N_2 = 9$) to those obtained from 30 min-heated ethanolic solutions. The maximum intensities were observed at the same λ_{exc} and λ_{em}, which shows that the new solvent system did not modify the chemical structure of the phosphorescent hydrolysis product of reserpine. Certainly, the aprotic nature of acetone does not promote reserpine solvation to the same extent as ethanol does. The interaction between the analyte and hydroxyl ions is, therefore, facilitated, which results in a faster reaction process.

The RTP intensity was then monitored as a function of acetone percentage. Reserpine was dissolved in solvent systems containing 8, 16, 30, 50 and 65% (v/v) of acetone in aqueous 0.4M NaOH. The highest signals were observed from 8, 16 and 30% acetone solutions. For these three systems, the I_A/I_B ratios were statistically similar ($P = 0.05$; $N_1 = N_2 = 9$). A 30% acetone solution was then employed as the solvent system for all further studies.

Heavy atom and surfactant effects

The effect of HA ions on the RTP emission of reserpine hydrolysis product was studied by testing four inorganic salts with general phosphorescence enhancers:[16,24,25] 1M NaI, 0.1M $TlNO_3$, 0.5M $Pb(NO_3)_2$ and 0.5M $AgNO_3$ solutions were prepared in ethanol–water (50:50 v/v) and spotted on the paper substrate prior to analyte deposition. The RTP signals in the presence of HA were then compared to those obtained in the absence of the inorganic salts.

Among the HA tested, only iodide and Tl(I) increased the RTP emission of the analyte. The enhancement factors are related in Table 2. NaDS increased the HA efficiency to a certain extent. Its effect, however, was not as remarkable as previously reported for other compounds,[23] where improvements of the order of four times were observed. This is an indicative that the presence of the HA itself raised the phosphorescence quantum yield of the analyte close to the maximum value.

Figure 2a shows the analyte-to-background signal ratio as a function of iodide concentration. The highest values were obtained with 1M and 2M HA solutions. At these concentrations, the paper is sufficiently coated to facilitate the interaction between iodide and analyte. At lower concentrations, the migration of HA to the interior of the paper diminishes the contact responsible for phosphorescence enhancement.[25] NaDS maximized the iodide efficiency when used at 0.02M concentration (see Fig. 2b). For surfactant concentrations higher than 0.05M, a reduction of the I_A/I_B ratio was observed. Since NaDS does not absorb in the excitation region of the analyte, the excess of surfactant on the surface of the paper probably diminishes the HA and analyte interaction.

Finally, no significant modifications were observed in the λ_{exc} and λ_{em} of the analyte. The highest intensities were detected at the same

Table 2. Heavy atom enhancement factors for reserpine hydrolysis product*

Heavy atom salt	$\lambda_{exc}/\lambda_{em}$† (*nm*)	Enhancement factor‡	
		No NaDS	NaDS
0.1M $TlNO_3$	303/465	1.4	2.0
1.0M KI	303/465	2.4	3.5

*Reserpine and surfactant concentrations were $10^{-4}M$ and 2%, respectively.

†Excitation (λ_{exc}) and emission (λ_{em}) maxima in the presence of HA salt.

‡Enhancement factors obtained on paper substrates with and without surfactant. The values were calculated dividing the net analyte signal in the presence and absence of HA. Six determinations of analyte signal and respective blanks were performed for each HA salt. The precision observed was within 10–15%.

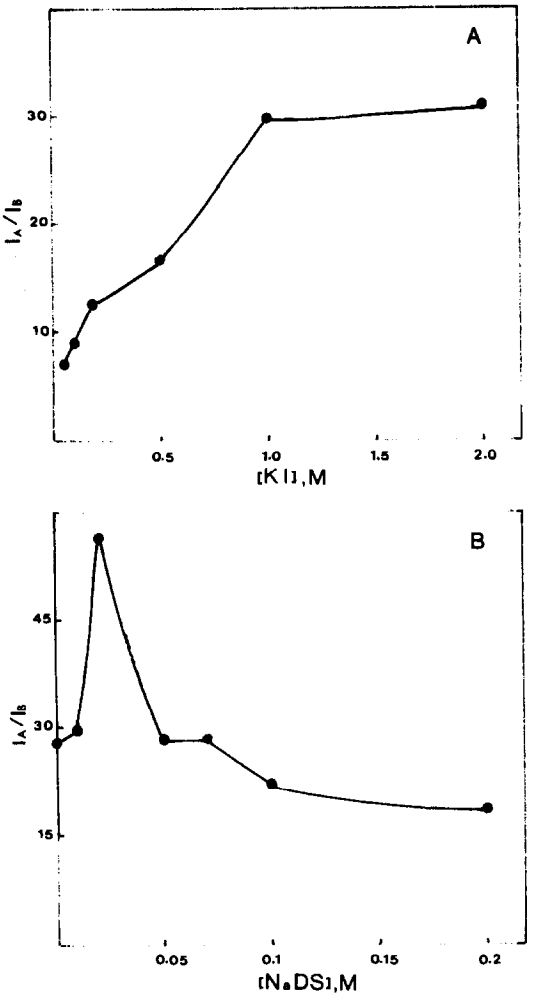

Fig. 2. Analyte-to-background signal ratio of a $10^{-4}M$ reserpine solution in acetone–aqueous $0.4M$ NaOH (30:70 v/v) *vs.* (A) KI molar concentration and (B) sodium dodecyl sulfate (NaDS) molar concentration in the presence of $1M$ KI.

wavelengths as those recorded in the absence of HA and NaDS within the variations of instrumental response (±3 nm).

Analytical figures of merit

The AFOM were obtained under experimental conditions for maximum RTP emission, $1M$ KI was employed as a phosphorescence enhancer in the presence of $0.02M$ NaDS. The calibration curve ($Y = 4.96 \times 10^6 X + 8.096$) showed a linear dynamic range of three orders of magnitude (10^{-7}–$10^{-4}M$), which was estimated by dividing the upper linear concentration by the limit of detection. The slope of the log–log plot was close to unity (0.92), indicating a linear relation between signal intensity and analyte concentration. The correlation coefficient of the analytical curve was 0.9993, showing a satisfactory precision (relative standard deviations varied from 10 to 15%). The limit of detection was calculated from the equation $c = 3s_b/m$, where the estimated background noise (s_b) from 16 determinations, the slope of the calibration curve (m), and $k = 3$ were employed.[26] For a 5 μl sample volume, the obtained value was 1.9 ng, which is approximately of the same order of magnitude as the one previously reported by SSRTP.[19]

Pharmaceutical formulation analysis

Reserpine pharmaceutical preparations usually contain additional compounds to complement hypertension treatment. These ingredients can interfere in the analysis of reserpine leading to inaccurate results.

Table 3 relates the RTP characteristics of some of those compounds obtained under experimental conditions for maximum reserpine phosphorescence emission. It is clear that only caffeine showed a considerable signal at the maximum λ_{exc} and λ_{em} of reserpine hydrolysis product. As expected, only caffeine increased the analyte signal of solutions prepared with ingredient concentrations 10× and 100× higher than reserpine concentration. The presence of this compound in pharmaceutical formulations will certainly interfere in the RTP analysis of reserpine. It is possible to avoid this effect by employing calibration curves that were obtained with standard solutions containing proper concentrations of caffeine.

The recovery of our method was tested by the analysis of two pharmaceutical formulations.

Table 3. RTP characteristics of active ingredients commonly encountered in reserpine dosage forms obtained in the presence of $1M$ KI and $0.02M$ sodium dodecyl sulfate

Compound*	$\lambda_{exc}/\lambda_{em}$† (*nm*)	I_A/I_B‡
Caffeine	275/439	17.7
Furosemide	311/458	1.0
Hydrochlorotriazide	308/468	1.1
Ascorbic acid	302/456	1.0

*$10^{-4}M$ solutions prepared in acetone–aqueous $0.4M$ NaOH (30:70 v/v).

†Excitation (λ_{exc}) and emission (λ_{em}) wavelengths at which the maximum intensities were observed.

‡The I_A/I_B ratios were measured at the maximum excitation (300 nm) and emission (468 nm) wavelengths of reserpine hydrolysis product. Six determinations of analyte signals and respective blanks were employed to calculate I_A/I_B. The precision was within 10–15%.

Gross[R] preparations contained reserpine as the only active ingredient while Adelfan–Esidrex[R] formulations also included hydrochlorotriazide and dihydralazine acid sulfate. Both preparations were analyzed by the calibration curve method. The recovery values were calculated based on six determinations of each sample solution. When the linear plot of reserpine standard solutions was used, a 98.7% recovery was obtained from Gross[R] tablets. The recovery from Adelfan–Esidrex[R] preparations, however, was below 90%, which is the lowest limit of the recovery range recommended by U.S.P. analysis guidelines.[20]

The low recovery was due to the presence of additional ingredients in the sample matrix. Hydrochlorotriazide and/or dihydralazine acid sulfate probably caused quenching of the analyte signal by inner filter effects.[10,14,19] As a tentative means of improving the final result, we prepared a new calibration curve with standard solutions containing hydrochlorotriazide, the only one of those ingredients available in our laboratory. The new recovery for Adelfan–Esidrex[R] tablets was 100.3%. Apparently, the presence of dihydralazine acid sulfate was not responsible for the low recovery initially obtained.

CONCLUSIONS

A rapid, simple and accurate method of analysis for reserpine commercial formulations has been reported. Phosphorescence emission was directly induced by reserpine dissolution in acetone aqueous 0.4*M* NaOH (30:70 v/v). This approach is convenient when compared to more tedious procedures commonly employed in fluorimetric methods. The use of low background paper pretreated with 1*M* KI and 0.02*M* NaDS resulted in a 1.9 ng limit of detection. Finally, satisfactory recoveries were obtained, even in the presence of ingredients capable of quenching the analyte signal. The necessity of previous separation steps of dosage forms containing this kind of ingredients can be avoided by the use of proper standard solutions.

Acknowledgements—This work was partially supported by Conselho Nacional de Desenvolvimento Cientifico e Tecnologico-CNPq. The authors also acknowledge Kathryn Norton for linguistic advice.

REFERENCES

1. R. E. Schirmer, *Analytical Profiles of Drug Substances*, Vol. 13, p. 737. Academic Press, Orlando, 1984.
2. L. S. Goodman and A. Gilman, *The Pharmacological Basis of Therapeutics*, 4th Ed. Macmillan, New York, 1970.
3. M. S. Karawya, A. A. Sharaf and A. A. Diab, *J. Assoc. Off. Anal. Chem.*, 1976, **59**, 795.
4. D. S. Mangala, B. S. Reddy and C. S. P. Sastry, *Indian Drugs*, 1984, **21**, 526.
5. A. Taira and D. E. Smith, *J. Assoc. Off. Anal. Chem.*, 1978, **61**, 641.
6. E. Thacker, *Anal. Proc.*, 1985, **22**, 136.
7. T. N. V. Prasad, E. V. Rao, C. S. P. Sastry and G. R. Rao, *Indian Drugs*, 1987, **24**, 398.
8. U. R. Cieri, *J. Assoc. Off. Anal. Chem.*, 1988, **71**, 515.
9. E. Dargel and J. B. Mielck, *J. Liq. Chromatogr.*, 1990, **13**, 3973.
10. M. I. Walash, F. Belal and F. A. Aly, *Talanta*, 1988, **36**, 731.
11. S. R. Varma, J. Martinez Catalayud and H. A. Mottola, *Anal. Chim. Acta*, 1990, **233**, 235.
12. J. Martinez Catalayud and C. Gomez Benito, *Anal. Chim. Acta*, 1991, **245**, 101.
13. T. Vo-Dinh, *Room Temperature Phosphorimetry for Chemical Analysis*. Wiley, New York, 1984.
14. R. J. Hurtubise, *Phosphorimetry: Theory, Instrumentation and Applications*. VCH, New York, 1990.
15. A. D. Campiglia and C. G. de Lima, *Anal. Chem.*, 1987, **59**, 2822.
16. T. Vo-Dinh and R. Hooyman, *Anal. Chem.*, 1979, **51**, 1915.
17. C. G. de Lima, M. M. Andino and J. D. Winefordner, *Anal. Chem.* 1986, **58**, 2867.
18. A. D. Campiglia and C. G. de Lima, *Anal. Chem.*, 1988, **60**, 2165.
19. E. Lue Yen and J. D. Winefordner, *Anal. Chim. Acta*, 1978, **101**, 319.
20. R. P. Bateh and J. D. Winefordner, *J. Pharm. Biomed. Anal.*, 1988, **1**, 113.
21. G. Ramis Ramos, M. C. Garcia Alvarez-Coque, A. M. O'Reilly, I. M. Khasawneh and J. D. Winefordner, *Anal. Chem.*, 1988, **60**, 416.
22. L. M. Perry, A. D. Campiglia and J. D. Winefordner, *Anal. Chem.*, 1989, **61**, 2329.
23. S. M. C. Gioia and A. D. Campiglia, *Anal. Chim. Acta*, 1994, **287**, 89.
24. T. Vo-Dinh, E. Lue Yen and J. D. Winefordner, *Anal. Chem.*, 1976, **48**, 1976.
25. M. M. Andino, M. A. Kosinski and J. D. Winefordner, *Anal. Chem.*, 1986, **58**, 1730.
26. G. L. Long and J. D. Winefordner, *Anal. Chem.*, 1983, **55**, 713A.