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Comparison of different phosphorimetric techniques for the determination of pindolol in multi-ingredient mixtures

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The sensitivity of luminescence makes it useful analytical tool for monitoring compounds of biochemical interest such as drugs. In this work we examined the possibility of the determination of pindolol in multi-ingredient mixtures by measuring the phosphorescence intensity at room temperature in aqueous, homogeneous micellar and mixed micellar solutions. The best working conditions in the investigated systems were proposed for the determination of investigated drug in different mixtures. The qualities and limitations of each system are discussed. The results obtained in this work indicate that the analytical procedures based on the use of room-temperature phosphorescence allow the determination of the pindolol in multi-ingredient mixtures.

Keywords: *pindolol · beta-adrenoacceptor blocker · MS-RTP · HAI-RTP · mixed micelles*

It is well known that nitrogen heterocyclic compounds play one of key role in processes of vital activity of human body. Owing to the high biological activity they are widely applied in medicine for treatment of various diseases. More than 60% of the most widespread synthetic preparations as an active component contain heterocyclic compounds. Pindolol (PIN) is a beta-adrenoacceptor blocker with wide variety of indications in medical practice, such as treatment of hypertantion, cardiac arrhythmia, angina and thyrotoxicosis. This compound act on the heart reducing the cardiac frequency, the contraction force and the cornary flow and very often abused in sports with little physical activity (fig.1) [1].

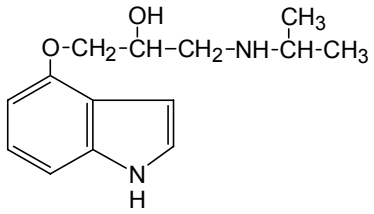

Fig.1. Structural formula of pindolol.

Luminescence analytical methods found wide application for the determination of pharmaceuticals in mixtures. Spectrofluorimetry is a powerful analytical technique for pharmaceutical applications due to its sensitivity and selectivity. The three-dimensional luminescence spectra, so-called excitation-emission matrix (EEM) and containing exhaustive information about mixtures under investigation, allows us to improve considerably of selectivity. However, the absolute elimination of overlapping spectra of individual components and simultaneous determination of all model compounds based on such approach are impossible in some cases. Room temperature phosphorimetry (RTP) has a number of preference for some applications in comparison with fluorimetry. It is well known that unlike fluorimetry phosphorescence provides two additional instrumental selectivity parameters, i.e. gating time and delay time, which can be used for improving of selectivity of component determination. Realization of RTP is based on observing the phosphorescence emission in solution at room temperature in the presence of heavy atom for increase of the rate of intersystem crossing and rigid molecular environment for enhancing of phosphorescence signal. The determination of the target component in the presence of fluorescing impurities by conventional fluorimetric procedures is commonly complicated by spectral overlaps, the absorption of the fluorescence of one component by the others, etc. A more reasonable technique consists in the determination of some nitrogen heterocyclic compounds on the basis of phosphorescence measurements, because the triplet states of the analyte molecules are more characteristic in comparison with the singlet states. The selectivity of the determination is substantially improved by the time selection of the analytical signal.

In some works [2–4], nitrogen heterocyclic compounds were determined in pharmaceutical preparations

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using the room-temperature phosphorescence method. However RTP determination of pindolol has not been examined in different media and the possibility of using this method for determination of pindolol in multi-ingredient mixtures has not been studied.

The aim of this work was to examine the possibility of the determination of pindolol in multi-ingredient mixtures by measuring the phosphorescence intensity at room temperature in aqueous, homogeneous micellar and mixed micellar solutions.

Materials and methods

Experimental

Solutions and reagents. In this work, we used pindolol (Wako, Japan), sodium sulfite, thalium nitrate, potassium iodide, Triton X-100 and sodium dodecylsulfate (SDS) (Acros, Germany). Solutions of sodium sulfite were prepared directly before measurements. Solutions were prepared using doubly distilled water obtained on a Milli-Q purification system (Millipore, France).

Instruments. The phosphorescence spectra of micellar solutions were recorded on Panorama (Lumex, Russia) and Shimadzu RF-5301 (Japan) spectrofluorimeters. Quartz cells (l = 1 cm) were used for experiments. The phosphorescence spectra of aqueous solutions were recorded on a Panorama spectrofluorimeter (Lumex, Russia) with quartz cells (l = 1 cm).

Chromatographic studies were performed on Shimadzu LC-10AT liquid chromatograph (Japan) with a UV detector and a Mightysil RP-C18 steel column with a size of 150×4.6 mm filled with an adsorbent with a particle size of 5 mm.

General procedure

Fluorimetry 0,1 ml aliquot of the stock standard solution of PIN with concentration $3 \cdot 10^{-4}$ M was placed into 10 ml calibrated flask and diluted to volume with water. The solution was thoroughly shaken.

MS-RTP (micellar-stabilized RTP) 0,1 ml aliquot of the stock standard solution of PIN with concentration $3 \cdot 10^{-4}$ M, 2,5 ml of 0,1 M SDS (or mixture of SDS and Triton X-100), 0,6 ml of 0,25 M $TlNO_3$ and 1 ml of 0,1 M sodium sulfite were placed into 10 ml calibrated flask and diluted to volume with water. The solution was placed in the ultrasound bath for 5 min.

HAI-RTP (heavy-atom induced RTP). 0,1 ml aliquot of the stock standard solution of PIN with concentration $3 \cdot 10^{-4}$ M, 0,66 g of solid potassium iodide and 1 ml of 0,1 M sodium sulfite were placed into 10 ml calibrated flask and diluted to volume with water. The solution was thoroughly shaken.

Results and discussion

Spectral characteristics

The rigid structures of some nitrogen heterocyclic compounds allow to receive well resolved spectra of luminescence at a room temperature. Three-dimensional data presentation gives more information about luminophore, including absorption and emission characteristics and also a direction of the maximal change of a signal. We present three-dimensional spectra of fluorescence (1) and phosphorescence (2) of pindolol in micellar solution (fig. 2). The choice of optimum values of excitation and emission wavelengths of individual compounds in this case substantially becomes simpler. The emission spectrum of fluorescence shows broads band with maxima at 308, 318 nm for PIN. The emission spectrum of phosphorescence shows broad band with maximum at 450 nm for PIN.

To record the phosphorescence spectra in aqueous solution, it is necessary to use an instrument with the time resolution of the signal (Panorama) because the phosphorescence intensity is lower by an order of magnitude without using micellar media. RTP spectra were recorded under the following scanning conditions: delay time – 40 ms and the gate time – 1000 ms. Under these conditions, only the phosphorescence spectrum is recorded, which significantly improves the selectivity of analysis. The shape of the spectra and the positions of the bands are analogous to those obtained in micellar solutions.

Influence of different media on phosphorescence intensity

Phosphorescence in micellar media. The generation of the RTP analytical signal usually requires the presence of micellar medium, the heavy atom and the absence of molecular oxygen. In micelle-stabilized RTP, it is common to use SDS as micellar agent and thallium nitrate as a heavy atom perturber. Choice of optimal conditions in the SDS medium was a subject of research in our previous work [4]. With increase of SDS concentration RTP intensity passes through a maximum. Probably, decrease in the intensity with further increasing of concentration can be explained by structural reorganization of formed micelles. Owing to change of physical and chemical properties of micelles at transition from spherical to lamellar form a decreasing of RTP signal is observed. Similarly, the increase of

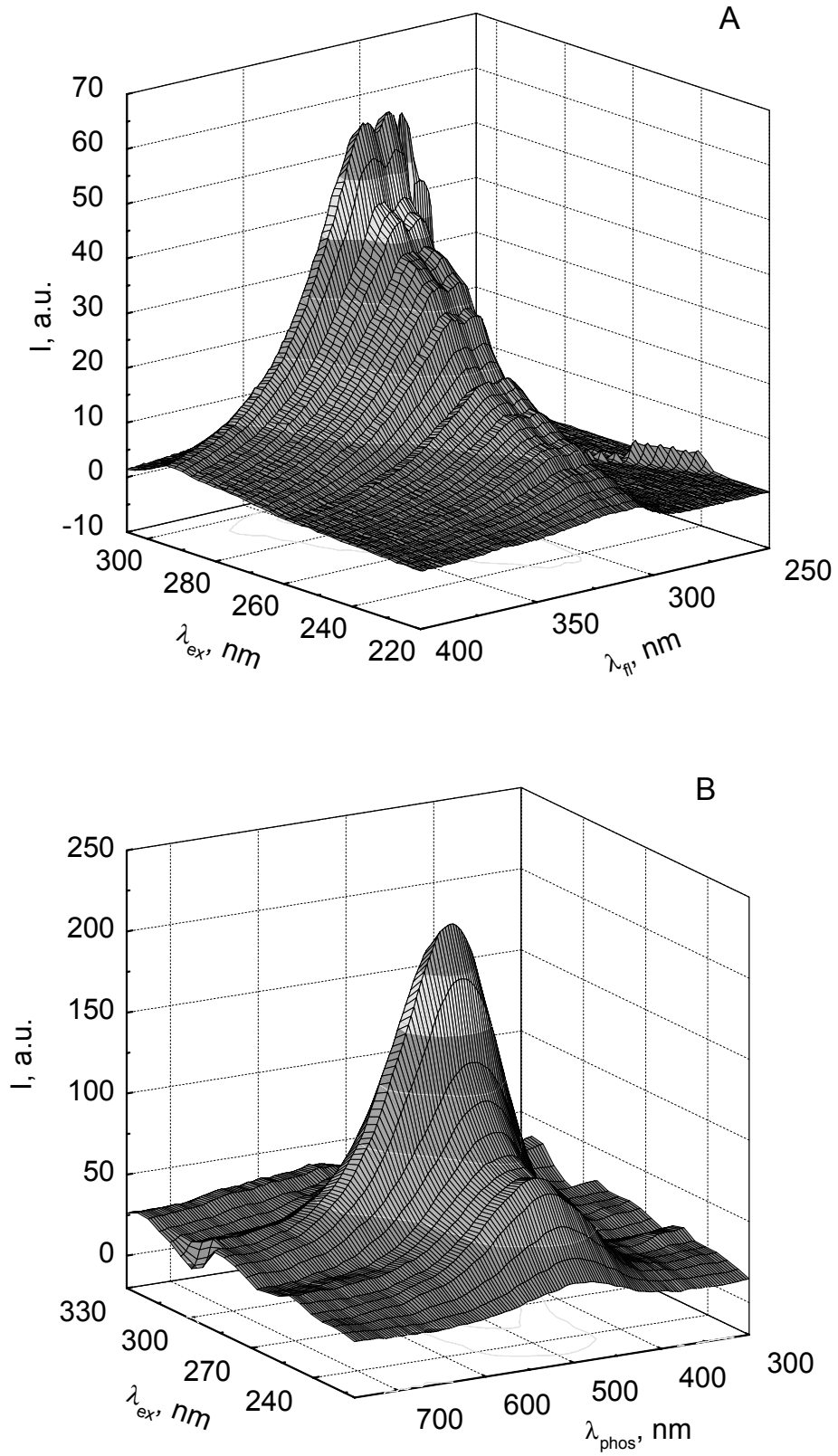

Fig. 2. Three-dimensional spectra of fluorescence (A) and phosphorescence (B) of PIN.

Table 1. Optimal values of pH and concentrations of reagents in different media

	Media	pH	C_{TlNO_3}, M	C_{KI},M	$C_{Na_2SO_3}$, M	C_{SDS}, M	$C_{Triton\ X}$, M
PIN	SDS	8–9	$1.75 \cdot 10^{-2}$	–	$7.0 \cdot 10^{-3}$	$2.5 \cdot 10^{-2}$	–
	SDS – Triton X	8–9	$1.5 \cdot 10^{-1}$	–	$1.0 \cdot 10^{-2}$	$2.5 \cdot 10^{-2}$	$1.0 \cdot 10^{-2}$
	aqueous	8–9	–	$6 \cdot 10^{-1}$	$1.0 \cdot 10^{-2}$	–	–

heavy atom perturber concentration gives an increase in phosphorescent intensity, while further concentration increasing results in decrease of RTP signal. So, there are a narrow optimal concentration range of SDS and thalium nitrate for phosphorimetric determination of pindolol. Table 1 presents the optimal conditions for the RTP measurements in SDS system.

It is already a known fact that different cationic and zwitterionic micelles alone do not allow to generate RTP signal and our results using CTAC and Triton X-100 have proved it. However, SDS and Triton X-100 form mixed micelles of homogeneous structure in wide range of concentrations [5]. Introducing different amounts of Triton X-100 in SDS solutions (containing thalium nitrate and sodium sulfite) the increase in intensity of a phosphorescence of PIN is observed (fig. 3) as well as increase of lifetime of molecules in triplet state. The observable increase in lifetimes is connected with decrease in efficiency of quenching by thalium ions. The increasing of Triton X-100 concentration reduce probability of meetings molecules of pindolol and thalium ions in mixed micelles. Probably processes of deactivation of

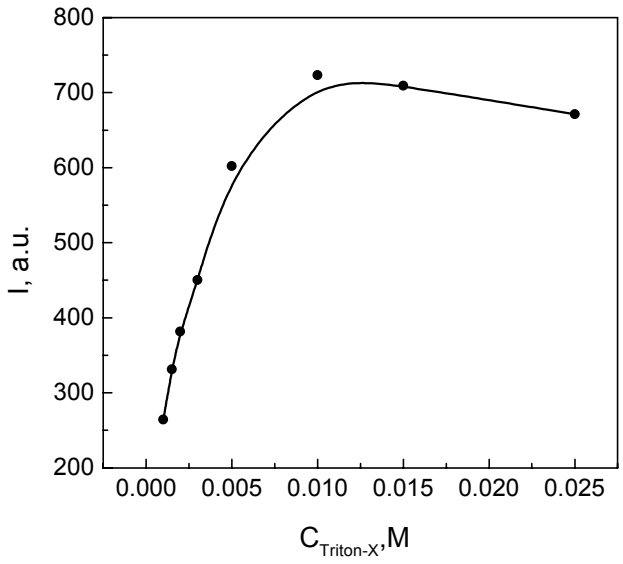

Fig. 3. The dependences of the phosphorescence intensities as a function of Triton-X concentration (C_{SDS} = 0.025 M).

energy of electronic excitation of molecules as a result of quenching by thallium ions of triplet states are defined by electrostatic interactions of thalium ions and mixed micelles. Mixed micelles allow to adjust a charge of micelle's surface and a degree of influence of the charged quenchers like thalium on triplet excited state of the molecule of interest. Using mixed micelles it is possible to select the proper ratio of SDS: Triton X-100 in order to obtain the maximal analytical signal for interested substance. Our results showed that the optimal ratio SDS : Triton X for pindolol is 2:1. Table 1 presents the optimal conditions of the RTP measurements in the systems under study.

Phosphorescence in aqueous media. The analysis of the data reported in the literature demonstrated that some groups of compounds, in particular, naphthalene derivatives and nitrogen heterocycles phosphoresce in the absence of organized media stabilizing triplet states [6–13]. In this case, the presence of a heavy atom perturber and sodium sulfite is a necessary condition for RTP generation. In literature this type of phosphorescence called heavy atom induced room temperature phosphorescence (HAI-RTP). As noted previously, for compounds containing amino groups in their structure the potassium iodide is frequently used as heavy atom perturber. In this case, an increase in the phosphorescence intensity can evidently be explained by the electrostatic interaction between the amino groups of the phosphor and the iodide ion [13]. Table 1 presents the optimal concentrations of the required reagents for obtaining the RTP signal in aqueous solutions.

RTP determination of pindolol in multi-ingredient mixtures

On the basis of the previously determined optimal conditions of the measurement of the analytical signal, calibration plots were obtained for the determination of the studied compounds. The performance characteristics are presented in Table 2. The direct determination of the studied component in mixtures was performed according to procedure described in Experimental (see the section General procedures). Results of the determination of

Table 2. Metrological characteristics of RTP method for PIN in different media (n=3, P=0,95)

	Media	Linear Range, M	Correlation coefficient	LOD, M
PIN	SDS	$7.0 \cdot 10^{-7} – 1.0 \cdot 10^{-5}$	0.997	$5.0 \cdot 10^{-7}$
	SDS – Triton X	$1.0 \cdot 10^{-7} – 2.0 \cdot 10^{-5}$	0.999	$9.5 \cdot 10^{-8}$
	aqueous	$9.0 \cdot 10^{-7} – 1.0 \cdot 10^{-5}$	0.994	$7.5 \cdot 10^{-7}$

Table 3. Determination of PIN in multi-ingredient mixtures (n = 5, P = 0,95)

Amounts of active components in mixture, mg	Found, %				
	Fluor	RTP_{aq}	RTP_{SDS}	$RTP_{SDS\text{-}TritonX}$	HPLC
pindolol, 10 *clopamide, 5*	107±3	96±3	97±3	99±3	95±5
pindolol, 10 *hydrochlorothiazide, 25*	109±3	95±3	98±3	95±3	96±6
pindolol, 5	98±2	96±3	98±3	98±3	94±6

PIN in mixtures using RTP in micellar and aqueous solutions are presented in Table 3.

Note that in the multi-ingredient mixtures fluorimetric technique (Fluor) has yielded the overestimated results in comparison with the declared value. Probably, the overestimated results are caused by a fluorescence of accompanying component.

For studying the effect of interfering compounds, the RTP determination was performed for each of these compounds (clopamide and hydrochlorothiazide) in micellar, mixed micellar and aqueous solutions. These components have no intrinsic RTP signal. Thus, the selective determination of pindolol in multi-ingredient mixtures is possible by using phosphorimetric techniques.

In selecting a procedure for phosphorimetric determination, the rapidity and cost of analysis should be taken into account along with the performance characteristics (the use of mixed-micellar solutions dramatically improves the sensitivity of the determination). However, the use of aqueous solutions for phosphorimetric measurements is characterized by a decrease in the total amount of reagents and the use of relatively lowly toxic potassium iodide as a heavy atom perturber. For this reason, we consider the measurement of the RTP signal without using organized media a more reasonable technique. The accuracy of the results of the determination of the pindolol in mixtures was verified by high-performance liquid chromatography (HPLC).

The results obtained in this work indicate that the procedures based on the use of room-temperature phosphorescence allow the determination of pindolol in multi-ingredient mixtures.

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