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Short Communication

Reversed-phase high-performance liquid chromatographic and derivative UV spectrophotometric determination of α-phenylethylamine in phosphomycin

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ABSTRACT

Two sensitive and selective methods were developed for the evaluation of α-phenylethylamine (α-PEA), an impurity possibly present in the antibiotic phosphomycin (PHO). The reversed-phase high-performance liquid chromatographic method uses a LiChrosorb NH_2 column with acetonitrile–0.1 *M* $NaH_2PO_4 \cdot H_2O$ buffer as eluent and allows the simultaneous detection of PHO and α-PEA in a complex matrix such as pharmaceutical preparations. The amine gives a linear detection response over the range 15–85 μg/ml. The derivative spectrophotometric method can specifically discriminate the amine in the range 230–280 nm. The α-PEA concentration is determined by measuring the distance between the maximum at 250 nm and the adjoining minimum at 248 nm in the second-derivative spectrum. A linear correlation was found in the concentration range 15–160 μg/ml. For both procedures, no sample pretreatment is required.

INTRODUCTION

(+)-α-Phenylethylamine (α-PEA) is used as a reagent in the synthesis of phosphomycin (PHO) to separate it, as (+)-α-phenylethylammonium (−)-*cis*-1,2-epoxypropylphosphonate, from its isomer [1,2]. For this reason, the antibiotic may contain this bioactive amine [3,4] as an impurity. Different methods, based on thin-layer chromatographic [5], gas chromatographic [6–8] and high-performance liquid chromatographic (HPLC) [9–11] techniques, are available for the simultaneous detection of this amine in fermented foods in which it occurs naturally. The methods proposed are generally complex and time consuming, and hence they are not suitable for the routine analysis needed for the quality control of pharmaceutical, parapharmaceutical and food products.

The aim of this investigation was to develop simple, high-resolution methods for the detection of α-PEA in raw materials and in pharmaceutical preparations containing PHO.

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EXPERIMENTAL

Reagents and chemicals

Sodium dihydrogenphosphate monohydrate ($NaH_2PO_4 \cdot H_2O$), sodium hydroxide and hydrochloric acid of analytical-reagent grade and acetonitrile of HPLC quality (LiChrosolv) were purchased from Merck (Darmstadt, Germany). Phosphomycin, calcium and disodium salts, and α-phenylethylamine were obtained from Sigma (St. Louis, MO, USA).

HPLC

All separations were carried out isocratically at room temperature using a Merck–Hitachi (Tokyo, Japan) Model 655A-11 liquid chromatograph, equipped with a Rheodyne (Cotati, CA, USA) Model 7125 injector (20-μl loop), a Model 655A variable-wavelength UV detector and a D2000 Chromato-integrator (Merck–Hitachi). A stainless-steel LiChrosorb NH_2 (particle size 10 μm) column (250 × 4 mm I.D.) and a LiChrocart NH_2 guard-column (4 × 4 mm I.D.) were obtained from Merck. The mobile phase was acetonitrile–0.1 *M* $NaH_2PO_4 \cdot H_2O$ buffer solution (pH 7.5) (35:65, v/v). Water for preparing buffer solutions was doubly distilled in an all-glass apparatus. All solvents were filtered through Millipore (Milford, MA, USA) HA filters (0.45 μm) and degassed ultrasonically before use. The flow-rate was 1.5 ml/min and the column effluent was monitored at 255 nm. Before analysis all samples were filtered through Millipore HV filters (0.45 μm).

Spectrophotometry

A Perkin-Elmer (Oak Brook, IL, USA) Model 552 double-beam spectrophotometer, a Model 561 recorder and 10-mm quartz cells were used. Normal and second-derivative parameters were range 380/190 nm, 1.0 ± 0.2 a.u.f.s., 0.5 response, scanning speed 120 nm/min and recorder chart speed 20 nm/cm.

Stock solutions

Standard solutions were prepared by dissolving 30–40 mg of α-PEA in a 50-ml volumetric flask with doubly distilled water. These solutions were then diluted to obtain solutions containing 5–200 μg/ml of the amine. Standard α-PEA solutions were employed to prepare synthetic mixtures containing PHO disodium salt at a concentration of 50 mg/ml. All freshly prepared solutions were stored at 4°C; under these conditions they were stable for 1 week.

Samples

PHO disodium salt. Approximately 2 g of each sample were dissolved in doubly distilled water and diluted to 50 ml.

PHO calcium salt. Approximately 2 g of each sample were dispersed in 45 ml of doubly distilled water, mixed thoroughly for 30 min and centrifuged; the supernatant was filtered through Whatman 40 paper and diluted to 50 ml.

Antibiotic tablets. Twenty tablets of each commercial product were finely powdered. Approximately 4 g of each sample were dispersed in 45 ml of doubly distilled water and treated as described for the preparation of PHO calcium salt samples.

Calibration

Stock solutions of α-PEA and α-PEA with PHO were used to construct the calibration graphs. Linear least-squares regression was used. Using a Cartesian coordinate system, the α-PEA concentrations (μg/ml) were plotted on the abscissa and the corresponding analytical responses on the ordinate. The amounts of the amine present were evaluated either as the peak areas, as estimated by the integrator, when the HPLC method was used, or the distances

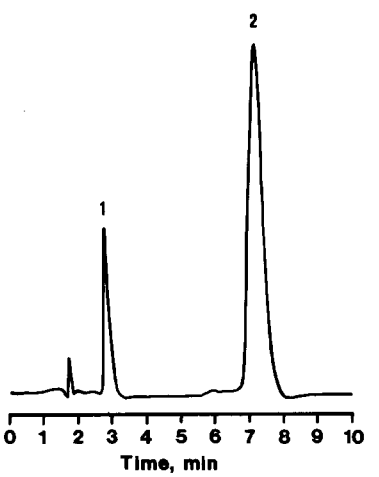

Fig. 1. RP-HPLC separation of (1) α-PEA (80 μg/ml) and (2) PHO (50 mg/ml).

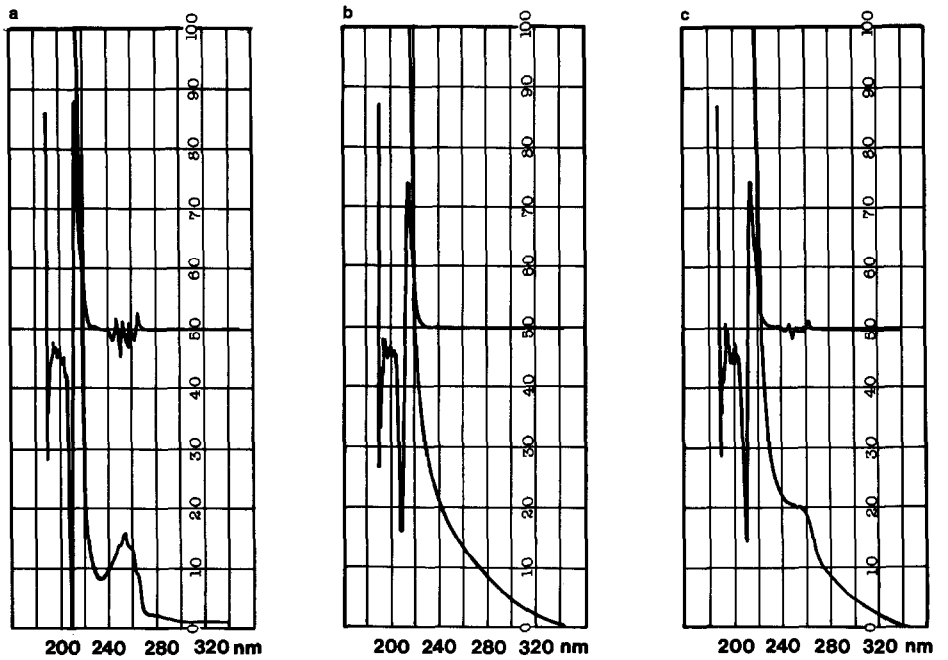

Fig. 2. Normal and second-derivative spectra of (a) α-PEA, (b) PHO and (c) a mixture of PHO (50 mg/ml) and α-PEA (0.1%).

measured as mm, between the maximum at 250 nm and the adjacent minimum at 248 nm, when the spectrophotometric method was applied. The calibration graphs constructed with α-PEA alone or in presence of PHO obeyed the same regression equations ($y = 199.315x$, $r = 0.999$, for the RP-HPLC method, and $y = 0.034x$, $r = 0.998$, for the spectrophotometric method). With both methods the intercepts did not differ statistically from zero.

RESULTS AND DISCUSSION

HPLC method

Fig. 1 shows the chromatograms obtained on analysing the standards of α-PEA and PHO. Statistical analysis did not reveal significant differences ($p < 0.01$) between the calibration graphs obtained using solutions containing the standard of amine alone or solutions containing the amine and a fixed

TABLE I

STATISTICAL ANALYSIS OF THE RESULTS OBTAINED ON ANALYSING A SYNTHETIC MIXTURE (50 mg/ml PHO)

Method	α-PEA concentration (μg/ml)	α-PEA found[a] (μg/ml)	Mean error (%)	S.D.[b]	R.S.D.[c] (%)
RP-HPLC	65.6	65.9	0.56	1.03	1.56
Second-derivative	68.0	68.7	1.03	1.25	1.82

[a] Mean of six determinations.
[b] S.D. = standard deviation.
[c] R.S.D. = relative standard deviation.

concentration of phosphomycin. A linear detection response was obtained between peak area and concentration of α-PEA over the range 15–85 μg/ml (corresponding to 0.3–1.7 μg injected), the detection limit for the impurity being 2 μg/ml (twice the signal-to-noise ratio). The accuracy and precision of the method are shown in Table I.

The method was used for the analysis of several commercial samples of PHO disodium and calcium salts and several pharmaceutical preparations containing the antibiotic. Only one sample of PHO calcium salt was shown to contain unquantifable traces of the amine.

Spectrophotometric method

Fig. 2a and b show the absorbance spectra and the second-derivative traces in the wavelength range 300–190 nm obtained for standards of α-PEA and PHO, respectively. The second-derivative UV profile of a mixture of the two compounds (0.1% α-PEA) is shown in Fig. 2c.

It is clear that the second-derivative method can discriminate the amine in the wavelength region 230–280.

Using this method there were no significant differences ($p < 0.01$) in the calibration graphs obtained with standard solutions of the amine and the amine plus an excess amount of the antibiotic. This confirms the absence of a matrix effect. A linear correlation was found in the concentration range 15–160 μg/ml. The detection limit was 4 μg/ml (twice the signal-to-noise ratio). The precision and the accuracy of this method in are reported Table I. The effectiveness of the resolution with this method was evaluated by analysing the same samples as tested with the HPLC method. The results confirmed the presence of trace amounts of the amine in one sample of phosphomycin calcium salt.

CONCLUSIONS

Two sensitive and specific methods have been developed for the accurate determination of α-phenylethylamine. Both methods are rapid and, further, they do not require preliminary extraction treatments; the samples are simply dissolved in water and the solutions must be filtered only if the matrix is not hydrosoluble. The HPLC method allows the simultaneous detection of PHO and its impurity in complex matrices such as pharmaceutical preparations.

The second-derivative spectrophotometric method is suitable for the determination of α-PEA in simple matrices. It is very rapid and does not involve destruction of the matrix, hence it is very useful for the validation of raw material batches or for stability studies.

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REFERENCES

1 E. J. Glamkowski, G. Gal, A. J. Davison and M. Sleztinger, *J. Org. Chem.*, 35 (1970) 3510–3512.
2 T. Otsuki, Y. Okamoto and H. Sakurai, *Synthesis*, (1983) 811–813.
3 J. Luthy and C. Schlatter, *Z. Lebensm.-Unters.-Forsch.*, 177 (1983) 439–443.
4 W. Lovemberg, *Toxicants Occurring Naturally in Foods*. Publication No. 1354, National Academy of Science, Washington, D.C., 1970.
5 K. D. H. Chin and P. E. Koeler, *J. Food. Sci.*, 48 (1983) 1826–1828.
6 W. F. Staruszchievicz and J. F. Bond, *J. Assoc. Off. Anal. Chem.*, 30 (1981) 584–591.
7 S. Yamamoto, H. Itano, H. Kataoka and N. Mekital, *J. Agric Food. Chem.*, 30 (1981) 435–439.
8 G. B. Baker, J. T. F. Wong, R. T. Coutts and F. M. Pasutto, *J. Chromatogr.*, 392 (1987) 317–331.
9 C. Buteau, C. L. Duitschaever and G. C. Ashton, *J. Chromatogr.*, 284 (1984) 201–210.
10 H. M. L. J. Joosten and C. Olieman, *J. Chromatogr.*, 356 (1986) 311–319.
11 M. A. J. S. Van Boekel and A. P. Arentsen-Stasse, *J. Chromatogr.*, 389 (1987) 267–272.