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# Multiwavelength spectrophotometric determination of acid dissociation constants
# Part III. Resolution of multi-protic ionization systems

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**Abstract**

A multiwavelength spectrophotometric (WApH) titration method was applied to study several multi-protic histamine $H_2$-receptor antagonists which involved four acid dissociation constants ($pK_a$ values) over the pH range of 2–10. Specifically, UV absorption spectra of the drug solution were acquired in the course of a pH-metric titration using an optical device based on a fibre optics dip probe, a light source and a diode array detector. Target factor analysis was utilized to deduce the $pK_a$ values from the spectral data recorded at different pH. It was noted that some of the $pK_a$ values were within mid pH range which were difficult to obtain because of insufficient absorption spectra acquired in the un-buffered region of the titration curve. With the aid of the WApH technique coupled with an optically transparent buffer, all $pK_a$ values have been successfully determined and were in excellent agreement with those measured using a conventional pH-metric method. 

*Keywords:* Spectrophotometric titration; pKa; Fibre optics; Target factor analysis; Histamine antagonists

## 1. Introduction

The acid dissociation constant ($pK_a$ value) is one of the key physicochemical properties that relates the rate and the extent of an ionizable drug compound entering the therapeutic target. Traditionally, pH-metric titration was employed for $pK_a$ determination in aqueous solution [1]. However, the success of this approach is sometimes hampered by poor aqueous solubility ($< 10^{-4}$ M). If the sample is soluble in water-miscible organic solvent, the apparent $pK_a$ values ($p_sK_a$) can be obtained pH-metrically. Extrapolation procedures such as the Yasuda–Shedlovsky method (a plot of $p_sK_a + \log[H_2O]$ vs. $A/\varepsilon + B$, where $[H_2O]$, $\varepsilon$, $A$ and $B$ represent, respectively, the molar concentration of water, the dielectric constant of the co-solvent mixture, the slope and the intercept of the plot) can be utilized to deduce the $pK_a$ values at zero organic solvent content [2,3].

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Spectrophotometric p$K_a$ determination is an alternative method provided that the compound is water soluble to the extent of $10^{-6}$ M and it contains chromophore(s) in proximity to the ionization centre(s). In our previous work [4], we devised a multiwavelength spectrophotometric (WApH) titration approach to interrogate drug compounds with one or two p$K_a$ values. Specifically, we employed a fibre optics dip probe, a UV light source and a photodiode array (PDA) detector in conjunction with a commercially available titrator (Sirius PCA101) to capture the absorption spectra of the sample in the course of a pH-metric titration. The target factor analysis (TFA) method was applied with success to deduce the p$K_a$ values of drug substances and resolve the absorption spectra of various species, without prior knowledge of their optical properties. In another study [5], we demonstrated that the TFA method outperforms the established first derivative technique in terms of obtaining p$K_a$ results. Moreover, the WApH technique has been implemented successfully to determine the p$K_a$ values of several water-insoluble pyridine derivatives which are difficult to measure pH-metrically [6].

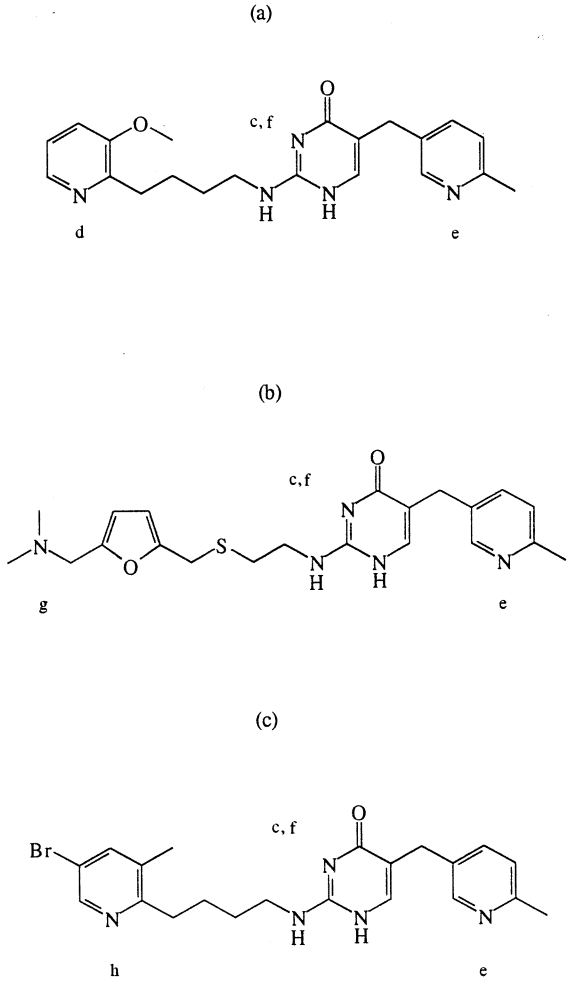


Fig. 1. Structures of the three histamine $H_2$-receptor antagonists: (a) icotidine, (b) lupitidine and (c) SK&F 93944 (the annotations are defined in Table 1).

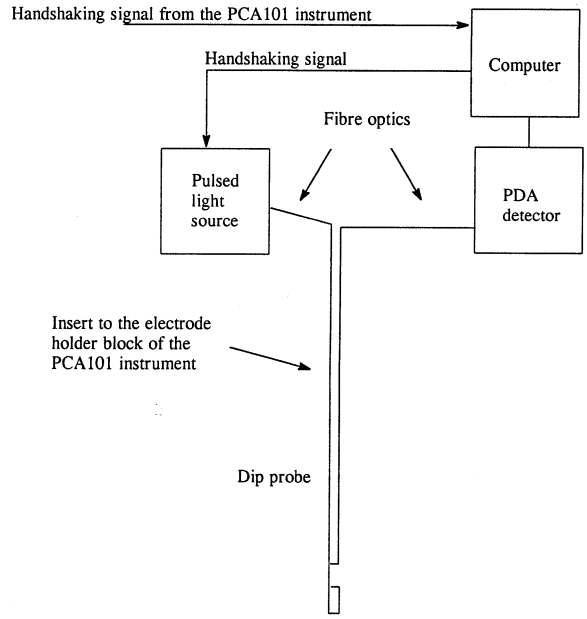


Fig. 2. Schematic for the experimental set-up used in the WApH titration.

In this work, we extend the WApH technique to examine three multi-protic histamine $H_2$-receptor antagonists, namely icotidine (SK&F 93319), lupitidine (SK&F 93479) and SK&F 93944. The structures of these compounds are given in Fig. 1. Here, we deliberately selected compounds with four p$K_a$ values and overlapping absorbing spectra to see whether the WApH technique is able to deduce all the unknown p$K_a$ values. It is noted that these p$K_a$ values may be difficult to measure by means of conventional spectrophotometric method using the spectral data obtained from a

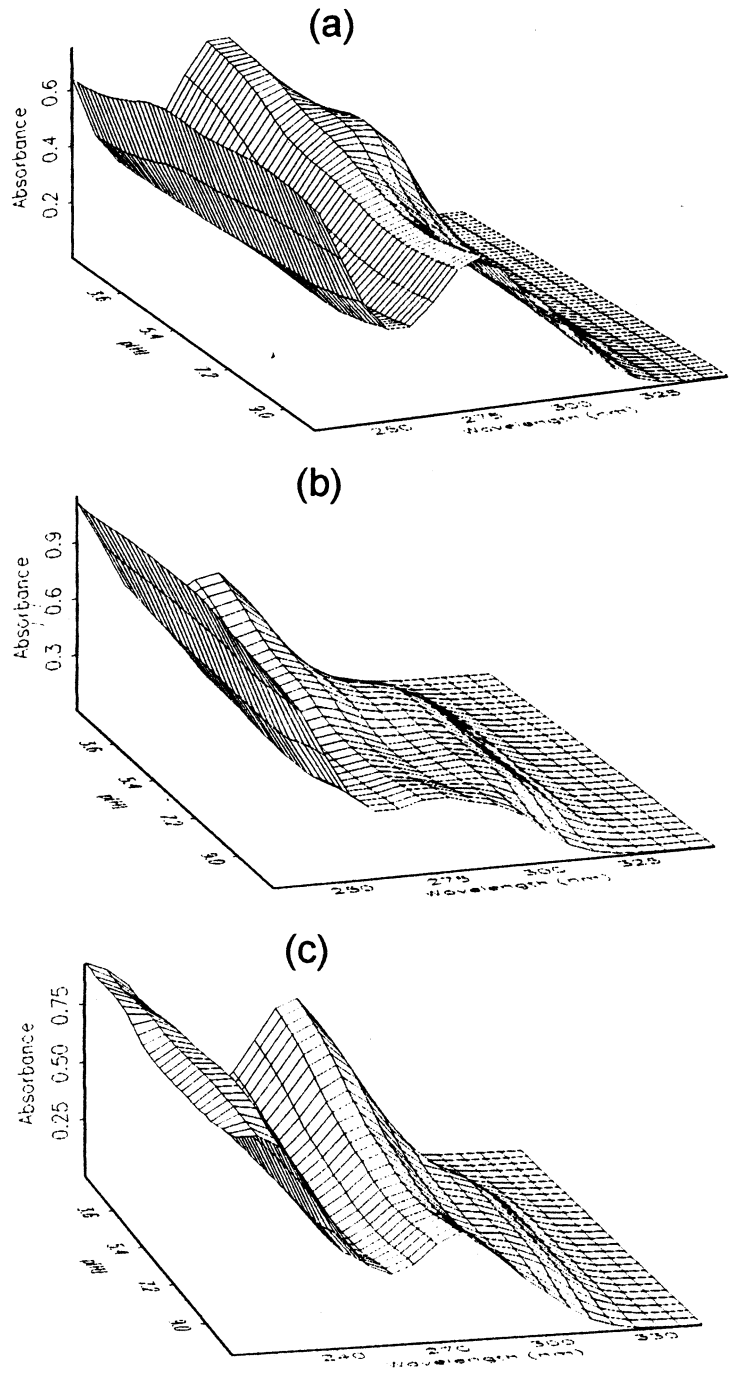


Fig. 3. Absorption spectra of (a) icotidine, (b) lupitidine and (c) SK&F 93944.

Table 1
p$K_a$ values of icotidine, lupitidine and SK&F 93944 as determined using the WApH and the pH-metric techniques at 25°C and an ionic strength of 0.15 M

| | Icotidine | | | Lupitidine | | | SK&F 93944 | |
|---|---|---|---|---|---|---|---|---|
| | WApH[a] | pH-metric[a] | Literature data [18][b] | WApH[a] | pH-metric[a] | Literature data [18][b] | WApH[a] | pH-metric[c] |
| p$K_{a,1}$ | $3.29 \pm 0.03$[d] | $3.32 \pm 0.02$ | 3.15 | $2.83 \pm 0.01$[d] | $2.79 \pm 0.01$ | 3.03 | $3.09 \pm 0.02$[d] | $3.04 \pm 0.05$ |
| p$K_{a,2}$ | $5.39 \pm 0.04$[e] | $5.40 \pm 0.01$ | ~5.60 | $5.93 \pm 0.01$[f] | $5.96 \pm 0.01$ | 6.11 | $4.06 \pm 0.02$[i] | $3.70 \pm 0.03$ |
| p$K_{a,3}$ | $6.22 \pm 0.06$[f] | $6.12 \pm 0.01$ | ~6.10 | $8.27 \pm 0.05$[h] | $8.25 \pm 0.01$ | 8.50 | $6.13 \pm 0.03$ | $5.72 \pm 0.03$ |
| p$K_{a,4}$ | $9.97 \pm 0.05$[g] | $9.92 \pm 0.01$ | 9.78 | $9.64 \pm 0.01$[g] | $9.66 \pm 0.01$ | 10.20 | $9.93 \pm 0.03$[g] | $9.85 \pm 0.05$ |

[a] Uncertainties equal to the standard deviation of the p$K_a$ values from three experiments.
[b] The p$K_a$ values were either estimated values or tabulated values of the corresponding ionizable groups.
[c] Values obtained using the Yasuda–Shedlovsky extrapolation method (uncertainty represents the estimated standard deviation).
[d] Isocytosine group (basic p$K_a$).
[e] 3-Methoxylpyridyl group.
[f] Picolyl group.
[g] Isocytosine group (acidic p$K_a$).
[h] Dimethylamino group.
[i] 3-Bromopyridyl group.

single analytical wavelength [1]. In the following discussion, a brief account of the TFA and the WApH methods will be given. It will be shown that the p$K_a$ values as determined using the WApH method are in good agreement with those obtained pH-metrically.

## 2. Method of calculations

In a WApH titration, the spectral data obtained is a series of spectra acquired at different pH values. The absorbance data matrix, **A**, can be expressed as follows:

$$\mathbf{A} = \mathbf{CE} \tag{1}$$

where **C** and **E** represent, respectively, the concentration–pH profile of the ionization system and the molar absorptivity matrix with the inclusion of the optical path length. The principal component analysis [7,8] is first applied to **A** to calculate an abstract solution for **C** and **E**, namely, $\mathbf{C}_{abs}$ and $\mathbf{E}_{abs}$, which contain only the primary eigenvalues ($\lambda_r$) and eigenvectors ($\mathbf{Q}_r$). The residual standard deviation [8], IND function [7,8], eigenvalue ratio [9] and reduced eigenvalue ratio [10,11] are utilized to identify the number of principal components (independent light absorbing species). In the TFA treatment, $\mathbf{C}_{abs}$ and $\mathbf{E}_{abs}$ are rotated to the solution with physical significant, $\mathbf{C}_p$ and $\mathbf{E}_p$, via a transformation matrix **T** defined as [8,12,13]:

$$\mathbf{T} = \lambda_r^{-1}\mathbf{C}_{abs}^{T}\mathbf{C}_t \tag{2}$$

$$\mathbf{A} \approx \mathbf{C}_{abs}\mathbf{T}\mathbf{T}^{-1}\mathbf{E}_{abs} \tag{3}$$

$$\mathbf{A} \approx \mathbf{C}_p\mathbf{E}_p \tag{4}$$

where the superscripts $-1$ and T denote, respectively, inverse and transpose operations. The test matrix $\mathbf{C}_t$ in Eq. (2) contains the concentration–pH profiles of the ionization system, which are generated theoretically [4]. In this study, the proton concentration is related to the operational pH reading by a multi-parametric equation [14].

The SPOIL function, as derived by Malinowski [8,13], is utilized to determine whether a test matrix is acceptable or not. In general, a test matrix in which the SPOIL function is minimized to a value not greater than 3.0 is considered as the solution for the target transformation procedure [4,8,12,13,15]. For a particular **A** matrix, the SPOIL function depends only on $\mathbf{C}_t$, which in

turn is a function of the sought p$K_a$ values. The TFA computation optimizes the p$K_a$ values for a global minimum of the SPOIL function. The SIMPLEX method [16] can be used for this purpose.

## 3. Experimental

Icotidine (SK&F 93319, trihydrochloride salt), lupitidine (SK&F 93479, trihydrochloride salt) and SK&F 93944 were provided by SmithKline Beecham Pharmaceuticals. Acetonitrile (far-UV grade) was supplied by Romil (Cambridge, UK). Potassium chloride and potassium dihydrogen phosphate (all AR grade) were obtained from Fisher (Loughborough, UK). Solutions were prepared in deionized water of resisitivity $>10^{14}$ Ω cm. The preparation and standardization of HCl and KOH solutions have been described elsewhere [14]. All titrations were performed by using a PCA101 or a GLp*K*a automatic titrator (Sirius, Forest Row, UK). The pH electrode was supplied by Orion (Ross™ type, Beverly, USA) and was calibrated titrimetrically in the pH range 1.8–12.2 in the relevant solvent media before use [2,3,14]. The processing of the pH-metric data, calculations of p$_s K_a$ values via a non-linear least-square procedure and Yasuda–Shedlovsky extrapolation treatments were carried out using *p*K*a*LOGP™ software (version 5.01, Sirius).

A schematic diagram for the WApH titration is given in Fig. 2. The optical system consists of a pulsed deuterium lamp (Cathodeon, Cambridge, UK) and a 256-element photodiode array (PDA) detector (Carl Zeiss, Herts., UK). This combination offers a spectral range of 200–735 nm with blaze wavelength at 220 nm. A bifurcated fibre optics dip probe (Custom Sensor & Technology, Missouri, USA) with optical path length of 1 cm is connected to the deuterium lamp and the PDA detector. Synchronization of the titrator, the pulsed deuterium lamp and spectrum acquisition by the PDA detector was accomplished using a terminate-and-stay-resident system [17]. The program for TFA treatment on the WApH data was coded in a TURBO C environment [4].

All titrations were performed in solutions of 0.15 M KCl under argon atmosphere at 25 ± 0.5°C using standardized 0.5 M HCl or 0.5 M KOH titrants. In the present study, sample concentrations of $3.7 \times 10^{-4}$ to $2.1 \times 10^{-3}$ M and $4.3 \times 10^{-5}$ to $5.0 \times 10^{-5}$ M were employed, respectively, for pH-metric and WApH titrations. In general, sample solutions of 10–20 ml volumes were pre-acidified to a reasonably low pH value (about 2.0) and then titrated alkalimetrically to an appropriate high pH value (about 10.5). The pH change per titrant addition was limited to about 0.1–0.2 pH units. pH data was acquired when the drift was less than 0.01 pH units per minute. For the WApH titrations, experiments were carried out in the presence of $2.5 \times 10^{-4}$ M potassium dihydrogen phosphate. Spectral data was recorded in the region of 210–350 nm after each pH measurement. As for the co-solvent experiments, a weighed amount of sample was dissolved in 15–40 wt.% of acetonitrile before titration.

## 4. Results and discussion

It was noted that icotidine and lupitidine are water-soluble up to a concentration of about 2 mM. This permits a direct comparison between the WApH and pH-metric techniques. However, the solubility of SK&F 93944 in water is relatively low. Therefore all pH-metric titrations for this sample were carried out using acetonitrile as co-solvent. The Yasuda–Sheldlovsky extrapolation procedure was invoked to deduce the p$K_a$ values at zero co-solvent content. In the view of the low sample concentrations used (about $10^{-5}$ M) in the WApH titrations, we introduced $2.5 \times 10^{-4}$ M potassium dihydrogen phosphate in the sample solution to provide buffering action in the mid range of pH such that the selected pH change per titrant addition could be maintained. We found that without this additional buffering action, it was not possible to acquire sufficient absorption spectra in the pH range 5–8 which led to difficulties in determining some of the p$K_a$ values (the dimethylamino group of lupitidine and the picolyl group of icotidine; see Fig. 1) as reported later.

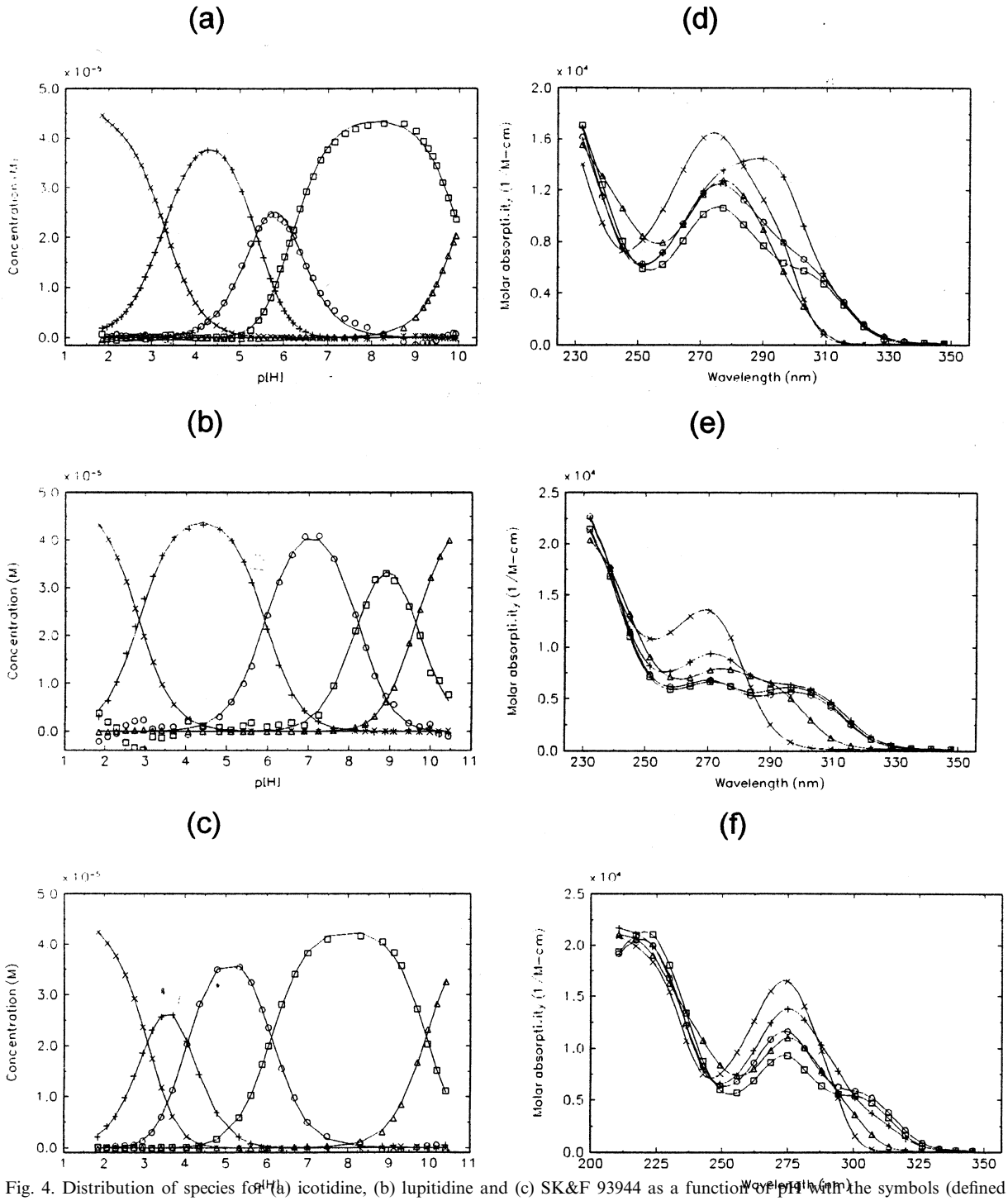


Fig. 4. Distribution of species for (a) icotidine, (b) lupitidine and (c) SK&F 93944 as a function of pH with the symbols (defined in Eq. (5)) represent the $\mathbf{C}_p$ matrix and solid lines denote the $\mathbf{C}_t$ matrix. Molar absorptivity coefficients of (d) icotidine, (e) lupitidine and (f) SK&F 93944. The symbols (defined in Eq. (5)) represent the elements in matrix $\mathbf{E}_p$. Solid lines are generated using the cubic spline interpolation method.

Fig. 3 shows the absorption spectra of icotidine, lupitidine and SK&F 93944. Principal component analysis revealed five independent components in each chemical system. The following ionization model is utilized in the pH-metric and TFA treatments to calculate the unknown p$K_a$ values

$$\underset{(\times)}{X_1} \overset{K_{a,1}}{\leftrightarrow} \underset{(+)}{X_2} \overset{K_{a,2}}{\leftrightarrow} \underset{(O)}{X_3} \overset{K_{a,3}}{\leftrightarrow} \underset{(\square)}{X_4} \overset{K_{a,4}}{\leftrightarrow} \underset{(\Delta)}{X_5} \quad (5)$$

where the symbols denote different UV active species and the term $X_5$ represents the fully deprotonated form of the drug. In Eq. (5), all charges and protons are omitted for clarity. Next, TFA was applied to these spectral data and in all cases, the unknown p$K_a$ values were successfully determined with the SPOIL function of each component less than 3.0. Table 1 lists the p$K_a$ values of icotidine, lupitidine and SK&F 93944 determined using the WApH and pH-metric methods. Note the good agreement between the two techniques and with the literature data, where available [18]. Fig. 4 depicts the distribution of species and the resolved molar absorptivity coefficients of icotidine, lupitidine and SK&F 93944. It can be seen that the TFA method is able to resolve the spectral data of the multi-protic ionization system even if the absorption spectra of individual species are very similar.

## 5. Concluding remarks

We have applied the WApH technique in conjunction with the TFA method to determine the p$K_a$ values of three histamine $H_2$-receptor antagonists. It has been demonstrated that this multi-wavelength approach is able to scrutinize drug compounds with more than two ionizable groups and overlapping absorption spectra. The p$K_a$ values, as determined using the WApH technique, are in excellent agreement with those measured pH-metrically.

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