Journal of Pharmaceutical and Biomedical Analysis 158 (2018) 236–246

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Multiwavelength UV-metric and pH-metric determination of the multiple dissociation constants of the lesinurad

Milan Meloun[a,*], Aneta Čápová[a], Lucie Pilařová[a], Tomáš Pekárek[b]

[a] Department of Analytical Chemistry, University of Pardubice, CZ 532 10 Pardubice, Czech Republic
[b] Zentiva k.s., U kabelovny 130, CZ 102 37 Prague, Czech Republic

ARTICLE INFO

Article history:
Received 14 March 2018
Received in revised form 23 May 2018
Accepted 29 May 2018
Available online 4 June 2018

Keywords:
Dissociation constants
Lesinurad
Spectrophotometric titration
pH-titration
REACTLAB
SQUAD84
ESAB

ABSTRACT

Potentiometric and spectrophotometric pH-titrations of the lesinurad for three consecutive dissociation constants determination were compared. Lesinurad is a selective inhibitor of uric acid reabsorption as part of a combination of medicines to treat high levels of uric acid in blood, also called hyperuricemia. Nonlinear regression of the pH-spectra with REACTLAB and SQUAD84 and of the pH-titration curve with ESAB determined three multiple close dissociation constants. The protonation scheme of lesinurad was suggested. A sparingly soluble anion L^- of lesinurad was protonated to the still soluble species LH, LH_2^+ and LH_3^{2+} in pure water. Three consecutive thermodynamic dissociation constants were estimated $pK^T_{a1} = 2.09$, $pK^T_{a2} = 4.25$, $pK^T_{a3} = 6.58$ at 25 °C and $pK^T_{a1} = 1.96$, $pK^T_{a2} = 4.16$, $pK^T_{a3} = 6.32$ at 37 °C by UV-metric spectra analysis. The graph of molar absorption coefficients shows that the spectrum of species LH_2^+ and LH vary in colour, while protonation of chromophore LH_2^+ to LH_3^{2+} has less influence on chromophores in the lesinurad molecule. Three multiple thermodynamic dissociation constants of 1×10^{-4} M lesinurad were determined by the pH-metric analysis $pK^T_{a1} = 2.39$, $pK^T_{a2} = 3.47$, $pK^T_{a3} = 6.17$ at 25 °C and $pK^T_{a1} = 2.08$, $pK^T_{a2} = 3.29$, $pK^T_{a3} = 6.03$ at 37 °C. The values of enthalpy $\Delta H^0(pK_{a1}) = 19.19\ kJ\ mol^{-1}$, $\Delta H^0(pK_{a2}) = 13.29\ kJ\ mol^{-1}$, $\Delta H^0(pK_{a3}) = 38.39\ kJ\ mol^{-1}$, show the dissociation process is endothermic. The positive values of $\Delta G^0(pK_{a1}) = 11.93\ kJ\ mol^{-1}$, $\Delta G^0(pK_{a2}) = 24.26\ kJ\ mol^{-1}$, $\Delta G^0(pK_{a3}) = 37.56\ kJ\ mol^{-1}$ at 25 °C indicate that the dissociation process of pK_{a2} is not spontaneous, which was confirmed by its value of entropy $\Delta S^0(pK_{a1}) = 24.37\ J\ mol^{-1}$, $\Delta S^0(pK_{a2}) = -36.79\ J\ mol^{-1}$, $\Delta S^0(pK_{a3}) = 2.79\ J\ mol^{-1}$. Three macro-dissociation constants of lesinurad and protonation locations were predicted by MARVIN and ACD/Percepta.

1. Introduction

Lesinurad is the urate transporter inhibitor for treating hyperuricemia associated with gout. The IUPAC chemical designation of lesinurad is 2–((5-bromo-4-(4-cyclopropylnaphthalen-1-yl)–4H-1,2,4- triazol-3–yl)thio)acetic acid with the molecular formula $C_{17}H_{14}BrN_3O_2S$ and there are various chemical names for lesinurad such as 878672-00-5; RDEA594; Zurampic; RDEA 594; UNII-09ERP08I3W. The molecular weight is 404.28 g/mol and water solubility is 0.00779 mg/mL. The registration number is CAS 878672-00-5 and PubChem CID 53465279, UNII 09ERP08I3W. Lesinurad has the trademark Zurampic (Fig. 1).

Gout is the most common inflammatory arthritis disseminated throughout the world [1]. Gout is caused by an increased level of the serum uric acid. It is three to four times more common in males than in women, and prevalence increases with age [2]. Increased concentrations of uric acid in the body result in the deposition of monosodium urate crystals, especially in joints. These crystals trigger the release of pro-inflammatory cytokines, especially interleukin(IL)-1-beta, which stimulates inflammation, and this results in great joint sensitivity and pain [3]. The acute stage is treated with short-term anti-inflammatory drugs, but long-term prevention can be achieved by lowering serum uric acid levels. Current urate-lowering drugs include both xanthine oxidase inhibitors and uricosuric agents. Recent advances in the role of urate transporters in kidney proximal tubules have resulted in the development of new generation uricosuric drugs including lesinurad [4]. Lesinurad belongs to the class of organic compounds known as phenyl-1,2,4-triazoles. It is a selective inhibitor of uric acid reabsorption which is used in combination with other agents in treating gout. It reduces serum uric acid concentration through the inhibition of an oral uric acid transporter 1 (URAT1) inhibitor, an enzyme

* Corresponding author.
E-mail address: milan.meloun@upce.cz (M. Meloun).

https://doi.org/10.1016/j.jpba.2018.05.047
0731-7085/

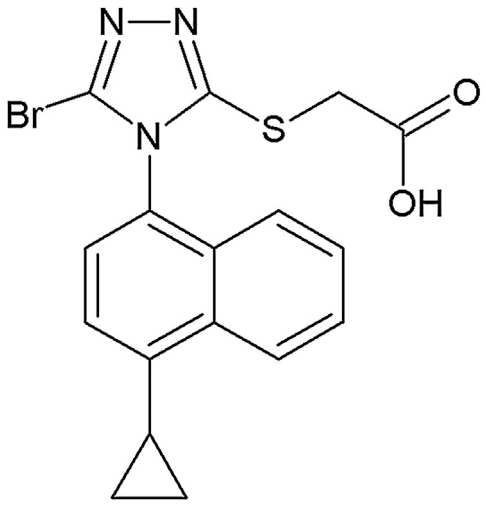

Fig. 1. Structural formula of lesinurad.

responsible for the reuptake of uric acid from the renal tubule, and OAT4, another uric acid transporter associated with diuretic-induced hyperuricemia. Lesinurad helps the kidneys remove uric acid from the body. Lesinurad 200 mg daily in combination with a xanthine oxidase is approved for urate-lowering therapy in patients with gout. Lesinurad combined with the xanthine oxidase inhibitor (XO) provides an effective treatment for gout-related hyperuricaemia. It was approved on December 2015 by the US Food and Drug Administration (FDA) and by the European Medicines Agency (EMA) in February 2016, ref. [5].

One of the most important physico-chemical characteristics of every drug is considered its pK_a value, which is a key physicochemical parameter influencing many biopharmaceutical characteristics in pharmacokinetic and bioavailability studies [6–9]. The dissociation constants of a compound influences lipophilicity, solubility, and permeability and plays a crucial role in the characterization of its absorption, distribution, metabolism and excretion (ADME) profile [10–14]. pK_a values can be either experimentally measured or theoretically predicted:

1. Spectrophotometry, and UV-metric spectra analysis (also called WApH technique [15]) in particular, is a highly sensitive convenient method to determine pK_a in very diluted aqueous solutions since it requires relatively simple equipment and can work with a sub-micromolar compound concentration (about 10^{-5} to 10^{-6} M), *cf. ref.*[16–18]. The authors [19–22] have shown that spectrophotometric titration in combination with suitable chemometric tools can be used to determine dissociation constants pK_a even for sparingly soluble drugs [20] [23]. The most relevant algorithms are SQUAD84 [17] and REACTLAB [24]. It is still believed that spectrophotometric data are inherently less precise than potentiometric data [25]; consequently most equilibrium constants are determined by means of pH-metric potentiometric titrations using ESAB [26] or HYPERQUAD [27,28].
2. The accuracy of theoretical pK_a predictions from a molecular structure with two predictive programs ACD/Percepta [29–35], MARVIN [29,31,32,34,36–39] was found to be the best of all nine similar programs.

The aim of our study was to examine the regression analysis of the pH-absorbance matrix with very small changes in spectra and to carry out a pH-metric potentiometric determination of the protonation model to find suitable conditions for a reliable regression determination of dissociation constants.

2. Computational details

Spectrophotometric pH-titration data were treated using the program SQUAD84 [17] and REACTLAB [24] which calculates equilibrium (protonation or dissociation) constants and molar absorptivities spectral profiles and distribution diagram of the relative concentrations of the pure species by the nonlinear regression of pH-spectra. A detailed tutorial of tittration also called the WApH technique [15], and alternative pH-metric titration have been was previously described [20,23].

3. Materials and methods

3.1. Materials

Lesinurad donated by ZENTIVA k. s., (Prague) with declared purity checked by a HPLC method and alkalimetrically, was always >99%. This drug was weighted straight to a reaction vessel resulting in a concentration of about 9.9×10^{-5} M. Other chemicals have been previously described [20].

3.2. Apparatus

The apparatus used and both titration procedures were described in detail [21–23,40]. The experimental and computation scheme to determine the dissociation constants of the multicomponent system is taken from Meloun et al., cf. page 226 in ref. [41] and all steps are described in details [21]. The free hydrogen-ion concentration [H^+] was measured on the digital voltmeter Hanna HI 3220 with a precision of ±0.002 pH using the combined glass electrode Theta HC 103-VFR. The potentiometric titrations of drugs with potassium hydroxide were performed using a hydrogen activity scale. Standardization of the pH meter was performed using WTW standard buffers values, 4.006 (4.024), 6.865 (6.841) and 9.180 (9.088) at 25 °C and 37 °C, respectively, in brackets.

3.3. Software

An estimation of the dissociation constants was performed by the nonlinear regression analysis of the UV-metric spectra analysis using SQUAD84 [17], REACTLAB [24] programs and potentiometric pH-metric titration data using the ESAB program [26,42], and by spectra interpretation using the INDICES program [43]. Most graphs were plotted using ORIGIN 9.1, ref [44]. ACD/Percepta [29–35] and MARVIN [29,31,32,34,36–39] programs for predictions of pK_a's are based on the structural formulae of drug compounds.

4. Results

The methods of analysis of the pH-absorbance response and pH-potentiometric titration curves have proven to be the best instrumental methods reliably determine close consecutive dissociation constants of even sparingly soluble drugs. Spectroscopic titration has been utilized as an alternative to potentiometric method to determine pK_a values of substances with large molar absorptivities due to its high sensitivity to concentrations of substance as low as 10^{-5} M. However, the examined compound must posses chromophore(s) in proximity to the ionization center(s) so that the protonated and deprotonated species exhibit sufficient spectral dissimilarity. The nonlinear regression analysis of spectrophotometric data is an effective and reliable tool, even in a case of small changes in spectra when changing the pH of the chromophore.