Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, Nos 8–12, pp. 1071–1074, 1990
Printed in Great Britain

0731–7085/90 $3.00 + 0.00

Short Communication

Dependence of the retention of pyrazolidine-3,5-diones on eluent pH in reversed-phase high-performance liquid chromatography*

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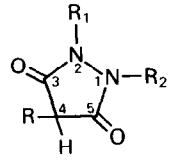
Keywords: *Reversed-phase HPLC; pH dependence of capacity factor; pyrazolidine-3,5-dione; phenylbutazone; oxyphenbutazone; ketazone; sulphinpyrazone.*

Introduction

In connection with the development of an analytical method for pyrazolidine-3,5-diones of pharmaceutical interest [1, 2], such as phenylbutazone (**I**), oxyphenbutazone (**II**), ketazone (**III**), and sulphinpyrazone (**IV**) (Table 1), an investigation of reversed-phase HPLC (RP-HPLC) retention characteristics of these compounds, which contain a mobile hydrogen in the 4-position, has been carried out. The previous work on the determination of pyrazolidine-3,5-dione drugs and their metabolites has been concerned mainly with the assay of biological samples [3–6]. Consideration of the various chromatographic systems mentioned indicate that most workers prefer to use RP-HPLC with a wide range of mobile phases, generally based on aqueous methanol or acetonitrile buffered at low or high pH. It would appear that the systems proposed have been selected somewhat arbitrarily since no clear explanation governing their choice is given.

It is well known that a change in pH that increases the ionization of a sample will also lead to reduced retention in a reversed-phase separation [7, 8]. The magnitude of solute retention is expressed by means of the capacity factor (k'), which is a measure of the stoichiometric mass distribution of solute between the stationary and mobile phases. Based on theoretical considerations, Horvath proposed equations [9] that have been used successfully

Table 1
Chemical structures of the pyrazolidine-3,5-diones



	R_1	R_2	R
Phenylbutazone **I**	C_6H_5	C_6H_5	C_4H_9
Oxyphenbutazone **II**	C_6H_5	C_6H_4OH	C_4H_9
Ketazone **III**	C_6H_5	C_6H_5	$CH_2—CH_2—CO—CH_3$
Sulphinpyrazone **IV**	C_6H_5	C_6H_5	$CH_2—CH_2—SO—C_6H_5$

*Presented at the "Second International Symposium on Pharmaceutical and Biomedical Analysis", April 1990, York, UK.

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in predicting the chromatographic behaviour of ionogenic compounds with reversed-phase systems. The relation between capacity factor of monoprotic acids and pH is given by the expression:

$$k' = \frac{k'_m + k'_a K_a/|H^+|}{1 + K_a/|H^+|},$$

where K_a and $|H^+|$ are the acidity constant of the solute and the concentration of the solvated proton in the mobile phase. k'_m and k'_a are defined as the capacity factors of molecular and anionic forms, respectively.

Therefore, if the pK_a values of the substances being investigated are known, by using only the retention data measured on the acidic and enolate forms as initial input in the Horvath model [9], it is possible to rapidly find the pH for the best separation in the shortest possible analysis time.

The present work was performed on an octadecylsilyl silica stationary phase with various methanol–water (6:4, v/v) eluents, covering a range of pH values, but with a constant ionic strength in order to compensate for any ionic strength dependence of retention [10].

Materials and Methods

Apparatus

A Perkin Elmer Tridet HPLC system equipped with UV (254 nm) and conductimetric detection was used with a Spherisorb ODS 5-μm column (21.5 nm × 4.5 mm i.d.). A S70 As/N Tacussel millivoltmeter with a Pt151 Tacussel Hydrogen electrode and Ag/AgCl reference electrode also was used.

Reagents

Phenylbutazone, oxyphenbutazone, sulphinpyrazone and ketazone were used without further purification. Identities were checked by IR and NMR spectra and purity by TLC.

The anionic forms were prepared by dissolving the parent compounds in chloroform and adding a molar equivalent of sodium ethoxide, filtering off the product, washing with chloroform and drying under vacuum.

Acetic, monochloracetic and dichloracetic acids and sodium sulphate were of analytical grade quality. Methanol was HPLC grade and water was double distilled prior to use. In order to set the apparent pH and the ionic strength of the mixed solvent system, the calculated quantities of acids and salts were dissolved in a given volume of methanol–water (6:4, v/v). After mixing, the phases were filtered on Millipore filter (0.65 μm).

Stock solutions

Stock solutions (6.0 mM) were prepared in methanol and diluted (1/50) with mobile phase prior to injection. Injection volumes of 20 μl were used throughout the investigation.

Procedures

Potentiometry. The pK_a^* values of the four compounds were measured by potentiometry in a galvanic cell with an hydrogen electrode versus Ag/AgCl reference in the mobile phase [methanol–water 6:4 (v/v), i.e. 54:46 (w/w), ionic strength 1.2×10^{-2} M]. The electrode was calibrated using IUPAC pH reference standards [11] with phthalate [12], oxalate and succinate buffers [13] in methanol–water 6:4 (w/w) and 5:5 (w/w). The e.m.f. measurements were performed on 10^{-3} M solutions of the molecular and anionic forms of each compound.

HPLC. All chromatograms were measured at 25°C using methanol–water 6:4 (v/v) as mobile phase at different pH values, if necessary the ionic strength was adjusted with sodium sulphate to $1.2\ 10^{-2}$ M. The capacity factors of the undissociated acids (k_m^*) were measured in a dichloracetic acid solution and the capacity factors of the conjugated bases (k_a^*) were measured on the anionic form in $0.4\ 10^{-2}$ M sodium sulphate. Retention times (t_r) were measured between the injection point and the peak maximum on the chromatogram. The void-volume time (t_o) was evaluated by injecting sodium sulphate (5×10^{-5} M) with conductimetric detection. Capacity factors were calculated using the expression $(t_r - t_o)/t_o$.

Results and Discussion

The pK_a^* values measured in methanol–water 6:4 (w/w) and 5:5 (w/w) were extrapolated to methanol–water 54:46 (w/w). The capacity factors of neutral and ionized species were determined by HPLC at constant ionic strength (see Table 2).

Figure 1 expresses the capacity factor according to Horvath equation under conditions of solute dissociation. At pH values <3,

Table 2
Acidity constants (pK_a) in methanol–water 5:5 (w/w) 6:4 (w/w) and 54:64 (w/w), i.e. 6:4 (v/v) and capacity factors (k') of the neutral and ionized species

Compound	pK_a^* Methanol–water % (w/w) 5:5	6:4	54:46	k' Methanol–water 6:4 (v/v) Molecular	Anionic
I	5.1	5.25	5.15	12.4	1.34
II	5.6	5.8	5.7	3.6	0.62
III	4.4	4.55	4.45	2.7	0.69
IV	3.4	3.5	3.45	6.0	1.12

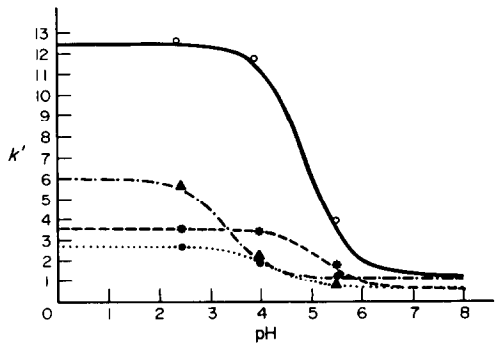

Figure 1
Theoretical curves and experimental values of the capacity factors versus the pH of the eluent: phenylbutazone (○) ——, oxyphenbutazone (*) ---, ketazone (●) ····· and sulphinpyrazone (▲) -·-·-.

the partitioning is only dependent on the relative solubility of the unionized forms of the drugs between the stationary phase and the solvent. These are directly related to the polarity of the R_1 and R_2 groups. When the pH is greater than this value, the partitioning becomes dependent also upon the drugs present in the ionized form. According to its high acidity ($pK_a^* = 3.5$), compound **IV** is ionized at lower pH than the other compounds. Nevertheless its higher molecular weight leads to a reduced solubility of the unionized form in the mobile phase than for compounds **II** and **III**. Consequently, the separation of the four compounds will be difficult at pH > 3, but as Fig. 1 predicts a good resolution is realised in a shorter time analysis for pH values between 5.5–6.1.

As an example three mobile phases A, B and C with pH* 2.55, 5.6 and 3.9, respectively were examined, and the experimental k' values reported in Fig. 1 were found to agree with the theoretical curves. As expected, the best separation occurs for a pH* near 5.6, where band spacing (Fig. 2) is good and the retention range is suitable. Alternatively, pH* = 2.55 also affords good band spacing with a longer time analysis, whereas C can be eliminated, as predicted.

Conclusion

The use of the Horvath model even in

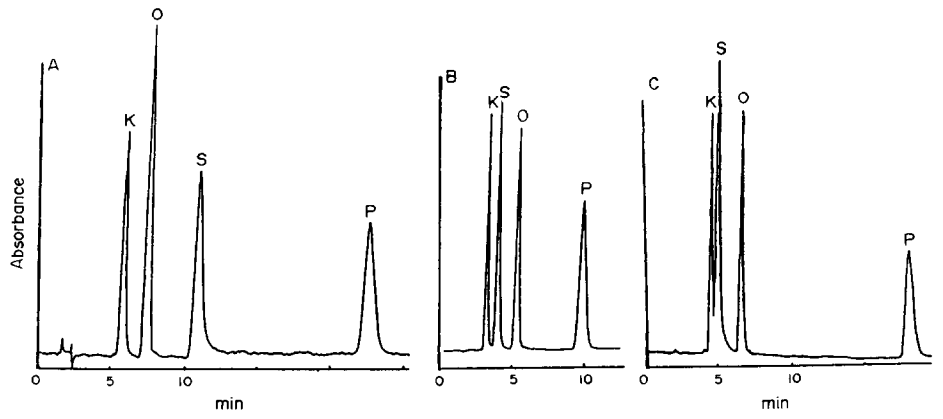

Figure 2
Chromatograms of pyrazolidine-3,5-diones: C_{18} column, flow rate, 1 ml min^{-1}; temperature, 25°C; sample component, phenylbutazone (P), oxyphenbutazone (O), ketazone (K) and sulphinpyrazone (S). Mobile phase methanol–water 6:4 (v/v). A, pH* = 2.55 (dichloracetic buffer); B, pH* = 5.6 (acetic buffer); C, pH* = 3.9 (monochloracetic buffer). Ionic strength 1.2 10^{-2} mol l^{-1}.

organic water mobile phase is a good way to find conditions for the rapid separation of monoprotic drugs.

Acknowledgements — The authors are grateful to the Ciba-Geigy company for generously providing samples.

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[Received for review 5 April 1990; revised manuscript received 3 July 1990]