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Ion-exclusion chromatography of aliphatic carboxylic acids on a cation-exchange resin by elution with polyvinyl alcohol

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Abstract

Ion-exclusion chromatography of aliphatic carboxylic acids of different acidity (pK_a) and hydrophobicity was investigated on a polystyrene–divinylbenzene (PS–DVB) based strongly acidic cation-exchange resin in the H^+ form and conductivity detection by elution with polyvinyl alcohol (PVA). When water was used as an eluent, the resolution of the carboxylic acids was very low and the peak accompanied a fronting depending on their hydrophobicities. Therefore, to improve the peak shape and the peak resolution, aqueous eluents containing PVAs (degrees of polymerization, n=500, 1500 and 2000) with many OH groups were tested for the ion-exclusion chromatographic separation of the carboxylic acids. When aqueous eluents containing PVA were used, the fronting was decreased dramatically by the effect of increased hydrophilicity of the PS–DVB cation-exchange resin surface due to adsorption of OH group in PVA. The high resolution ion-exclusion chromatographic separation without the fronting and highly sensitive conductimetric detection of the carboxylic acids was accomplished successfully by elution with a 0.2% PVA (n=1500)–10% methanol–water.

Keywords: Mobile phase composition; Carboxylic acids; Polyvinyl alcohol

1. Introduction

Ion-exclusion chromatography is a useful technique for the separation of organic and inorganic weak acids, especially those of a hydrophillic nature. Several reviews have been published on ion-exclusion chromatography [1–9]. Typically, a high-capacity cation-exchange resin in the H^+ form is used in ion-exclusion. When water was used as an eluent, peaks with a leading front edge (fronted peaks) were obtained for carboxylic acids such as valeric acid with hydrophobic nature [4,6,8]. This is a result of a adsorption as a side effect in ion-exclusion chromatography. Accordingly, an aqueous solution of a strong acid such as sulfuric acid is generally used as the eluent for the separation of weak acids [1,2]. This is done to repress the ionization of the sample acids and to ensure that they are entirely in their molecular forms. However, when conductivity detection is used, the acidic eluent causes a rather high background conductance and reduces the ability to detect sample acids.

In a previous paper [8], we have reported an ion-exclusion chromatography of aliphatic carboxylic acids by elution with sugars and polyols of low background conductivity. By using sugar or polyol eluents, highly sensitive conductimetric detection and high resolution for aliphatic carboxylic acids without fronting has been accomplished. The improvement of fronting is based on the effect of increased hydrophilicity of the cation-exchange resin surface by the adsorption of sugars or polyols.

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Additionally, Morris and Fritz [7] have reported that the use of *n*-butanol as an eluent is very effective for the ion-exclusion chromatographic separation of carboxylic acids on a sulfonated polymeric resin with conductimetric detection.

A major goal of this research was to find an eluent that gives sharp chromatographic peaks for aliphatic carboxylic acids with some hydrophobicity, instead of sugar or polyol eluents. In the present work, high-performance separation and highly sensitive conductimetric detection was achieved for aliphatic carboxylic acids on a polystyrene–divinyl benzene (PS–DVB)-based strongly acidic cation-exchange resin in the H^+ form by elution with polyvinyl alcohol (PVA). As is well-known, the PVA has many –OH groups with hydrophillic nature in the molecule.

It is shown that excellent resolution and very sensitive conductimetric detection of aliphatic carboxylic acids is obtained using a 0.2% PVA (degree of polymerization=1500)–10% methanol–water as the eluent.

2. Experimental

2.1. Apparatus

The ion chromatograph consisted of a Tosoh CCPD metal-free eluent delivery pump at a flow-rate of 1 ml/min (Tokyo, Japan) equipped with a Reodyne 7125 sample injector of a 100 μl PTFE loop.

Conductimetric detection was carried out with a Tosoh IC-8010 equipped with a constant temperature controller at 35°C. A Tosoh SC-8010 chromatographic data system was used for instrument control and for data collection and processing.

2.2. Column

The separation column was a Tosoh glass column (300 mm×8 mm I.D.). The column was packed by the slurry packing technique and equilibrated through with the eluent before each chromatographic run.

2.3. Resin

A Tosoh TSKgel SCX PS–DVB-based sulfonated strongly acidic cation-exchange resin in the H^+ form with particle size of 5 μm and and exchange capacity of 4.2 mequiv./g was used for all chromatographic runs.

2.4. Reagents and solutions

Standard solutions of aliphatic carboxylic acids and inorganic acids were prepared as the acid from reagent-grade chemicals without further purification. The aqueous eluents containing PVAs were prepared as 0.5% solutions by dissolving the PVA (degrees of polymerization=1000, 1500 and 2000) in distilled, deionized hot water.

PVAs and organic solvents used as organic modifier were obtained from Wako (Osaka, Japan).

3. Results and discussion

3.1. Effect of PVA on ion-exclusion chromatographic separation of carboxylic acids

As shown in Fig. 1, although the carboxylic acids are separated from each other depending on the pK_a values and the hydrophobicity by elution with water, the resolution is very low and the peak is fronted due primary to the hydrophobic adsorption effect as a side effect in the ion-exclusion chromatography [4,5,8].

In an attempt to reduce peak fronting, PVAs with different degrees of polymerization (n=500, 1500 and 2000) were tested and the elution performance were compared for the separation of various carboxylic acids. As is well-known, the PVAs have a different number of alcoholic OH groups in the molecules with hydrophillic property.

As shown in Fig. 2A–C, the preliminary experiment showed that all PVA eluents are applicable to the ion-exclusion chromatographic separation of carboxylic acids. The obtained ion-exclusion chromatographic chromatograms show complete ion-exclusion chromatography separation of the carboxylic acids from strong acid (sulfuric acid) by elution with 0.2% PVAs–water. As can be seen from the peak

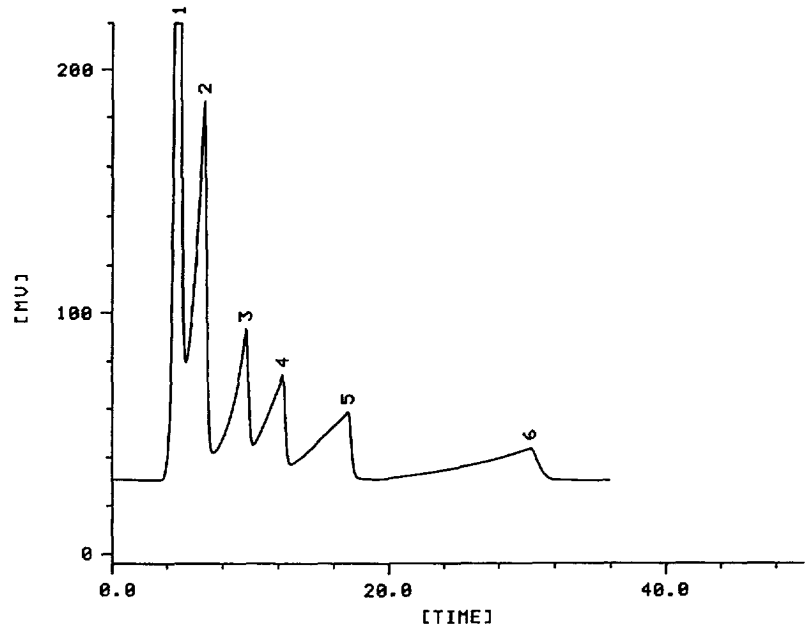

Fig. 1. Ion-exclusion chromatogram of sulfuric acid and some carboxylic acids by elution with water. Separation column: TSKgel SCX strongly acidic cation-exchange resin in the H^+ form (300 mm×8 mm I.D.), column temperature: 35°C, eluent flow-rate: 1 ml/min, sample concentration: 0.5 m*M*, conductimetric detection sensitivity: 1 μS/cm=10 mV. (1) Sulfuric acid, (2) formic acid, (3) acetic acid, (4) propionic acid, (5) butyric acid, (6) valeric acid.

shape of valeric acid, fronting was dramatically decreased by alcoholic OH group with hydrophillic nature in the PVA. This means that the hydrophobicity of the PS–DVB-based cation-exchange resin surface is decreased by the adsorption of the PVA. Thus, it is possible to modify the polarity of cation-exchange resin surface by the adsorption with the PVA. The above result indicates that the PVA additive coats the resin surface by a dynamic equilibrium between the liquid phase and the resin surface. The OH groups of the PVA make the resin surface more hydrophillic.

3.2. Effect of PVA concentration on peak symmetry

The peak symmetrical factor in the concentration range of 0–0.5% PVA ($n=500$) was determined using valeric acid with the largest fronting of carboxylic acids. The peak symmetrical factor was defined as the ratio of time of leading front edge to peak top (A) and time of peak top to tailing rear edge (B) on base line for peak of valeric acid. That is, the peak symmetrical factor (A/B) is 1 for a completely symmetrical peak, >1 for a fronting peak and <1 for a tailing peak.

As shown in Fig. 3, the peak symmetrical factor of valeric acid on elution with water was an extremely large value (ca. 15.5). However, the symmetrical factor decreased drastically toward ca. 1 by increasing concentration of PVA in the eluent. Since the symmetrical factor was ca. 1 between 0.2 and 0.5% PVA, the optimal PVA concentration for the ion-exclusion chromatographic separation of carboxylic acids was judged to be 0.2% in terms of the peak symmetry.

3.3. Effect of PVA concentration of retention volumes of carboxylic acids

As can be seen from Fig. 4, the retention volumes (V_R) of all carboxylic acids gradually increased by increasing concentration of PVA ($n=500$) ranging from 0–0.2% in the eluent. Finally, the V_R of carboxylic acids was constant above 0.2% PVA in the eluent. This means that the optimal PVA concentration for the ion-exclusion chromatographic

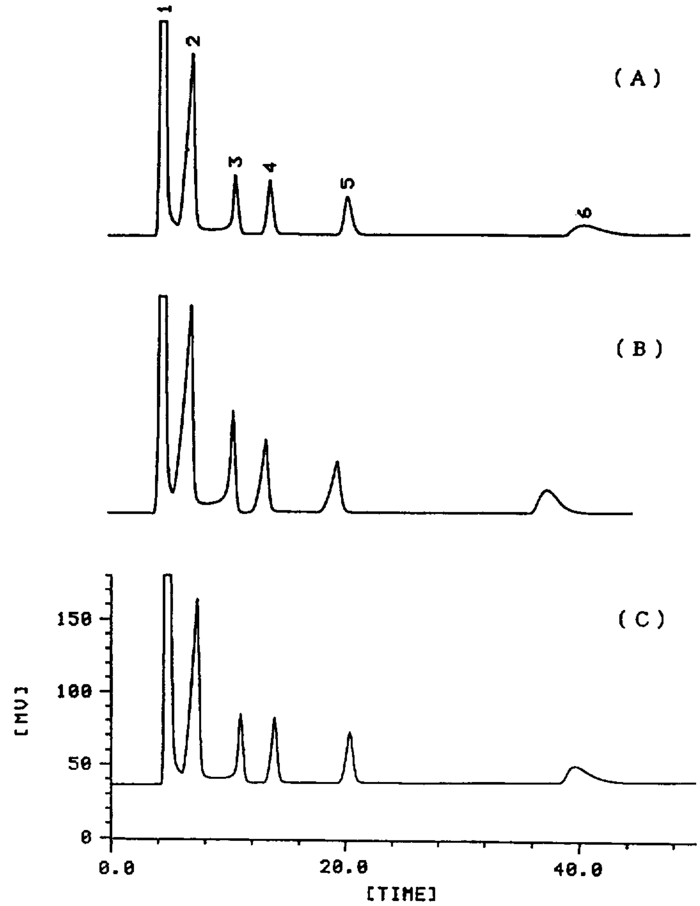

Fig. 2. Effect of degree of polymerization (n) of PVAs on the ion-exclusion chromatographic separation of carboxylic acids. (A) $n=500$ (eluent background conductivity=43 μS/cm), (B) $n=1500$ (eluent background conductivity=19 μS/cm), (C) $n=2000$ (eluent background conductivity=24 μS/cm). Eluent concentration: 0.2% PVAs. Other chromatographic conditions and peak identities as for Fig. 1.

separation of carboxylic acids is ca. 0.2%. Similar results to those with PVA eluent have been obtained on ion-exclusion chromatography of carboxylic acids by elution with sucrose having 8 alcoholic OH groups [8].

3.4. Effect of degree of polymerization of PVA on the ion-exclusion chromatographic separation of carboxylic acids

As described in Section 3.3 all of PVAs was applicable as an eluent for the ion-exclusion chromatography separation of carboxylic acids. However, the solubility of each PVA to water is a very important factor for the preparation of eluent. As is well-known, the solubility is in the order of PVA ($n=500$)>PVA ($n=1500$)>PVA ($n=2000$).

On the other hand, the eluent background conductivity due mainly to the presence of ionic impurity in PVAs was 43 μS/cm for PVA ($n=500$), 19 μS/cm for PVA ($n=1500$) and 24 μS/cm for PVA ($n=2000$).

From the above results, when considering the peak resolution, the eluent background conductivity and water solubility, the best choice of eluent for the ion-exclusion chromatographic separation of carboxylic acids was concluded to be PVA ($n=1500$).

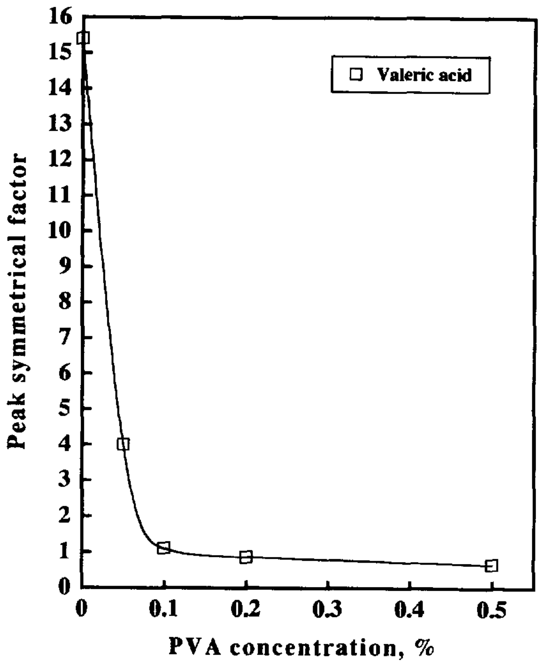

Fig. 3. Effect of PVA concentration on peak symmetrical factor for valeric acid R.S.D. with much higher hydrophobic property. Other chromatographic conditions as for Fig. 2. PVA: $n=500$. The peak symmetrical factor (A/B) is defined as time of leading front edge to peak top (A) and peak top to tailing rear edge (B) on base line for peak of valeric acid.

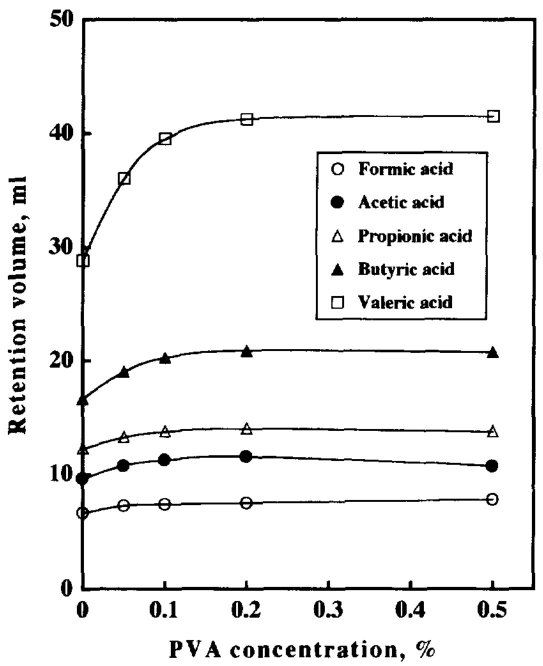

Fig. 4. Effect of PVA concentration on retention volume (V_R) of carboxylic acids. PVA: $n=500$. Other chromatographic conditions as for Fig. 3.

3.5. Effect of organic modifiers

As described in our previous papers [4,6,8], addition of organic solvent (organic modifier) to the eluent might be expected to reduce the adsorption effects of hydrophobic carboxylic acids such as propionic, butyric and valeric acids to the PS–DVB cation-exchange resin surface and thus decrease their V_R values. Therefore, the effect of the concentration of organic modifier such as methanol and acetonitrile in the eluent on the V_R values of carboxylic acids was examined. This was proven experimentally, where the organic modifiers were added to the 0.2% PVA ($n=1500$) eluent.

As shown in Fig. 5, the remarkable decrease in V_R values of the hydrophobic carboxylic acids such as butyric and valeric acids by addition of organic modifier to the PVA eluent might be due mainly to the effect of adsorption of methanol on the polymeric resin surface as the same as the result of ion-exclusion chromatography by elution with n-butanol–water [7].

A similar result to methanol–water eluent was also obtained on ion-exclusion chromatography of carboxylic acids by elution with PVA–water eluent.

A reasonable separation and conductimetric detection of carboxylic acids was accomplished by elution with 0.2% PVA–10% methanol, as shown in Fig. 5C.

3.6. Calibration graph

A calibration graph was obtained by plotting peak area against carboxylic acids in the concentration range of 0–2 mM by elution with 0.2% PVA ($n=1500$)–10% methanol–water. The calibration graph obtained was non-linear at higher concentration of above ca. 1 mM. This might be due mainly to decrease in the degree of dissociation of carboxylic acids at higher concentration.

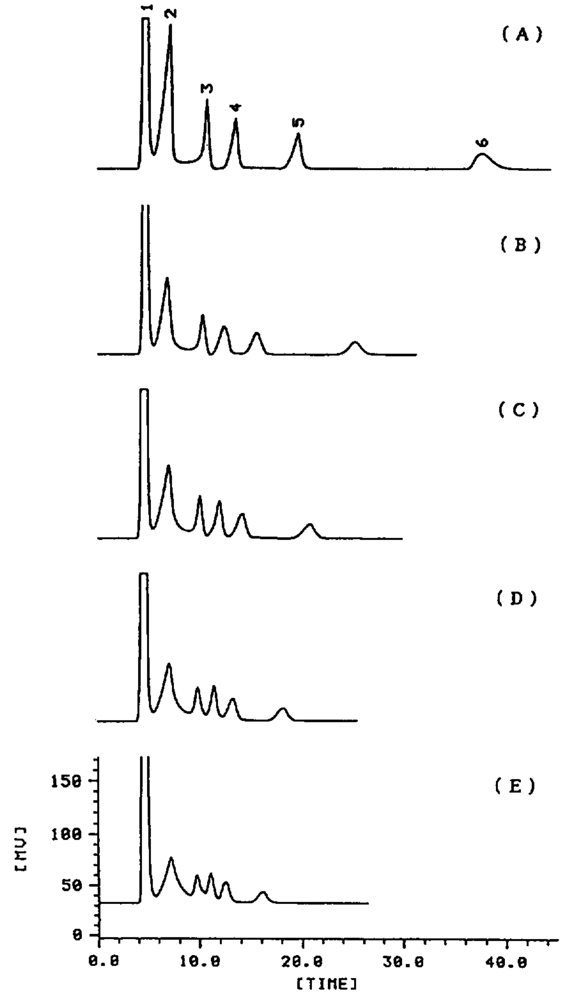

Fig. 5. Effect of methanol concentration in 0.2% PVA on the ion-exclusion chromatographic separation of sulfuric acid and some carboxylic acids. (A) 0% methanol, (B) 5% methanol, (C) 10% methanol, (D) 15% methanol, (E) 20% methanol. Other chromatographic conditions and peak identities as for Fig. 4.

Therefore, in order to obtain a linear calibration graph, a conductivity enhancement column in the Li^+ form was connected in series after the separation column [10].

Fig. 6A and B show the conductimetric ion-exclusion chromatograms of sulfuric acid carboxylic acids with and without the conductivity enhancement column. The reasonable separation and detection was obtained by conductivity enhancement method. The small negative conductivity response between acetic and propionic acids was caused by water in sample solution.

The calibration graph of carboxylic acids obtained by conductivity enhancement method was linear over the concentration range of 0–ca. 2 m*M* carboxylic acid because their Li salts are completely ionized.

3.7. Reproducibility

The reproducibility (relative standard deviation, $n=6$) of carboxylic acids obtained by repeated injections made of a 0.5 m*M* standards were 0.039–0.12% for V_R and 0.12–0.41% for the peak area.

3.8. Detection limits

The detection limits of carboxylic acids at $S/N=3$ by elution with 0.2% PVA ($n=1500$)–10% methanol–water are listed in Table 1 together with the data reported previously for water and sucrose–methanol–water eluents [8].

Since this method uses a non-acidic eluent, the eluent background conductivity was much lower (ca. 9 μS/cm) than that in a 0.5 m*M* sulfuric acid eluent (ca. 390 μS/cm) by conventional ion-exclusion chromatography. Therefore, the noise level on the eluent background conductivity was as low as water or sucrose– methanol–water eluents [8]. Since there is no ionization suppression of carboxylic acids by H^+ ion in the strongly acidic eluent, the detector response was as highly sensitive as the water or sucrose–methanol eluents.

4. Conclusion

In this work, simple and highly sensitive way for the ion-exclusion chromatography of aliphatic carboxylic acids was investigated using a strongly acidic cation-exchange resin in the H^+ form and PVA–methanol–water eluent.

A good resolution without fronting and highly sensitive conductimetric detection of carboxylic acids was obtained by elution with a 0.2% PVA–10% methanol–water. Due to relatively low eluent background conductivity (~9 μS/cm), the detection

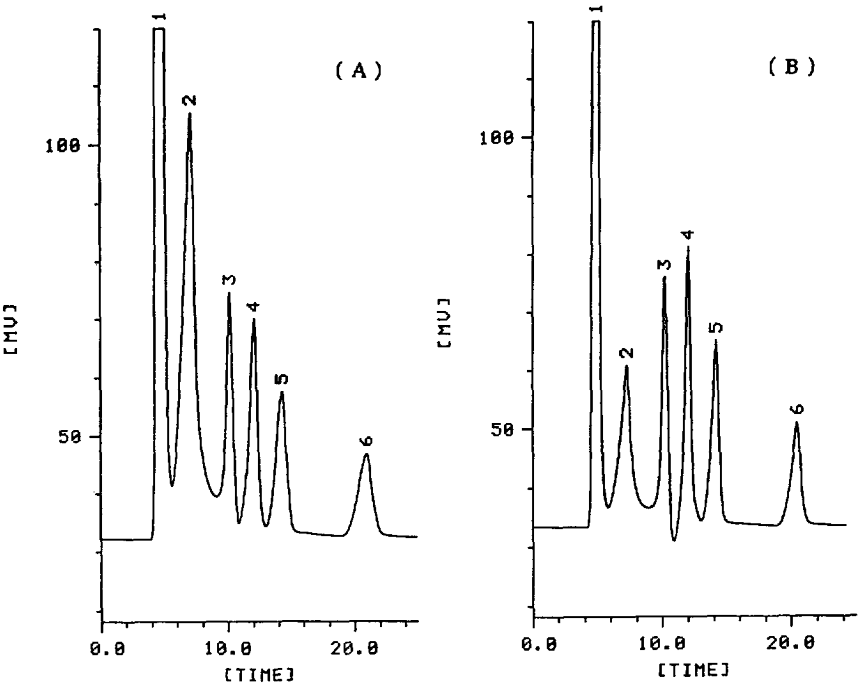

Fig. 6. Ion-exclusion chromatograms of strong acid and some carboxylic acids by elution with 0.2% PVA–10% methanol–water with (A) and without (B) conductivity enhancement column in the Li^+ form. Conductivity enhancement column: TSKgel SCX in the Li^+ form (100 mm×8 mm I.D.). Other chromatographic conditions and peak identities as for Fig. 5.

limits of carboxylic acids were as low as those of water eluent.

Although a high resolution separation of carboxylic acids was accomplished by this method, the time to separate completely carboxylic acids was long (~ca. 25 min). Therefore, the development of high-resolution ion-exclusion chromatography of carboxylic acids in a short-time (ca. 10 min) by using a small separation column will be required and will be the subject of future work.

Table 1
Detection limits of some aliphatic carboxylic acids at signal/noise=3 on elution with PVA–methanol–water (A), sulfuric acid–water (B), water (C) and sucrose–methanol–water (D)

Carboxylic acid	Detection limit (μM)			
	A	B [8]	C [8]	D [8]
Formic	0.48	8.4	0.12	1.40
Acetic	0.73	84	0.27	2.50
Propionic	0.96	150	0.40	3.44
Butyric	1.26	330	0.70	4.43
Valeric	2.22	1200	1.16	6.88

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