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The determination of oxalic acid, oxamic acid, and oxamide in a drug substance by ion-exclusion chromatography

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Abstract

Oxalic acid, oxamic acid and oxamide are potential impurities in some active pharmaceutical ingredients (API). The retention and separation of oxalic and oxamic acids are particularly challenging using conventional reversed-phase HPLC due to their high polarity. An ion-exclusion chromatography (IEC) method has been shown to provide good separation and sensitivity for the three oxalate-related impurities in a hydrophobic API matrix. The method uses a Dionex IonPac ICE-AS1 column with 95/5 (v/v) 0.1% sulfuric acid/acetonitrile as the mobile phase and UV detection at 205 nm. Development and validation of this method are described.

Keywords: Oxamic acid; Oxalic acid; Oxamide; Ion-exclusion chromatography

1. Introduction

Ion-exclusion chromatography (IEC) is a technique for separating small neutral and weakly acidic or basic compounds based on a Donnan exclusion mechanism [1–6]. It is particularly useful for the separation of small organic acids using a strong cation exchanger. At a sufficiently low pH, weak organic acids are undissociated, or weakly dissociated, and can diffuse into the resin pores on the stationary phase, while strongly anionic substances are rejected by the resin. The IEC retention of organic acids depends primarily on the dissociation of the acid (elution in the order of pK_a values) [7,8]; it is also affected by adsorption partitioning of the acid with the stationary phase [8]. In this paper, the IEC technique is applied to the determination of oxalic acid, oxamic acid, and oxamide (Fig. 1) in a hydrophobic drug substance matrix.

Oxalic acid, oxamic acid, and oxamide can occur as impurity products in some pharmaceutical synthetic processes. These oxalate-related compounds are highly polar and poorly UV-absorbing. Both oxalic and oxamic acids elute close to the solvent front under reversed-phase high-performance liquid chromatography (HPLC) con-

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ditions. With normal-phase conditions, the two acids interact with the column strongly, resulting in poor resolution and peak shape. Publications are available regarding the separation of oxalic acid from other organic acids [7–13]; however, in many cases, oxalic acid is poorly retained and data is not readily available on the separation of oxalic acid and oxamic acid. Although ion-exchange chromatography methods have shown good retention of oxalic acid [14,15], inclusion of oxamide, a neutral species would make the separation difficult because of the lack of retention of neutral species with ion-exchange chromatography (IEC). In this paper, the separation of the three oxalate-related compounds using an IEC method is compared with the results using various reversed-phase and normal-phase HPLC methods. The validation of the IEC method is described for the quantitation of oxalic acid, oxamic acid, and oxamide at relatively low levels (approximately 0.5–20 μg/ml for oxalic and oxamic acids, and 0.2–6 μg/ml for oxamide, which are equivalent to 0.1–4 and 0.04–1.2%, respectively, in a sample solution of 0.5 mg/ml) in a drug substance matrix.

2. Experimental

2.1. Chemicals

Acetonitrile (ACN), hexane, and isopropyl alcohol (IPA) of HPLC grade, sulfuric acid (H_2SO_4, 95–98%, w/w) and acetic acid of GR grade were purchased from EM Science, Gibbstown, NJ. Oxalic acid (99%), oxamic acid (98%), and oxamide (98%) were purchased from Aldrich, Milwaukee, WI. Trifluoroacetic acid (TFA) was purchased from Mallinckrodt, Paris, KY. Phosphoric acid (H_3PO_4), HPLC grade, was purchased from Fisher, Pittsburgh, PA. The API matrix was from Eli Lilly, Indianapolis, IN. Water was deionized and filtered through a Milli-Q™ water purification system from Millipore, New Bedford, MA.

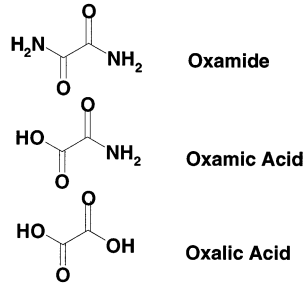


Fig. 1. Structures of oxalic acid, oxamic acid, and oxamide.

2.2. Instrumentation

Chromatographic analyses were carried out using either a Hewlett-Packard HP-1100 or a Waters™ 600E liquid chromatograph equipped with an autosampler and a UV–Vis detector. Chromatograms were recorded and processed on an in-house chromatography data acquisition system.

2.3. IEC method conditions

The Dionex IonPac ICE-AS1 (9 × 250 mm, 7.5 μm particle) column was purchased from Dionex, Sunnyvale, CA. The optimum mobile phase was 5/95 (v/v) ACN/0.1% H_2SO_4. The flow rate was 0.8 ml/min. The sample was prepared in 30/70 (v/v) ACN/H_2O. The injection volume was 10 μl. The detection wavelength was 205 nm.

2.4. Columns for method screening

Other columns that were tested for method screening are summarized in Table 1.

3. Results and discussion

3.1. Method development

3.1.1. Comparison of screening methods and results

A variety of conditions were screened using reversed-phase, normal-phase, and ion-exchange chromatography for the separation of oxalic acid, oxamic acid, and oxamide.

Most evaluations were conducted under reversed-phase HPLC conditions, using columns that had demonstrated good separation of certain polar molecules. As summarized in Table 2, these reversed-phase conditions generally gave poor

Table 1
Columns used for method screening

Column	Manufacturer
IonPac CS14, 250 × 4 mm, 7.5 μm	Dionex, Sunnyvale, CA
SynchroPac SCD-100, 150 × 4.6 mm, 5 μm	Micra Scientific, Northbrook, IL
Zorbax SB-C8, 250 × 4.6 mm, 5 μm	Hewlett Packard, San Fernando, CA
Zorbax CN, 150 × 4.6 mm, 5 μm	Hewlett Packard, San Fernando, CA
Zorbax SB-CN, 250 × 4.6 mm, 5 μm	Hewlett Packard, San Fernando, CA
Platinum C8 EPS, 250 × 2.1 mm, 5 μm	Alltech, Deerfield, IL
Hypercarb, 100 × 4.6 mm, 7 μm	Alltech, Deerfield, IL
Inertsil C4, 250 × 4.6 mm, 5 μm	GL Sciences, Shinjuku, Tokyo, Japan
Kromasil C1, 250 × 4.6 mm, 5 μm	MetaChem Tech., Torrance, CA
Aquasil C18, 250 × 4.6 mm, 5 μm	Keystone Scientific, Bellefonte, PA
Supelco discovery, RP-amide C16, 250 × 50 mm, 5 μm	Supelco, Bellefonte, PA
AquaSep C8, 250 × 4.6 mm, 5 μm	ES Industries, West Berlin, NJ

peak shape and poor retention of oxalic acid and oxamic acid or interference from the solvent front.

To increase the retention of the two acids and to alter the selectivity, normal phase HPLC was also investigated. The two acids were retained strongly on the column until very high percentages of IPA (approximately 80%) in hexane were used. The peaks of the two acids were very broad and could not be resolved. These results are summarized in Table 3.

Finally, ion-exclusion conditions were evaluated, and the results are listed in Table 4. Broad peaks and poor resolution of oxalic acid and oxamic acid were observed with a Dionex IonPac CS14 column, which was most often used for cation-exchange separations. On the other hand, excellent separation and peak shape for all three oxalate-related compounds were obtained using a Dionex IonPac ICE-AS1 column, which was designated for ion-exclusion separations. Fig. 2 shows typical chromatograms using the IonPac ICE-AS1 column.

Table 2
Reversed-phase HPLC screening conditions and results

Column	Mobile phase	Results
Synchro Pac SCD-100	5/95 ACN/pH 2.0 H_3PO_4	Poor separation and peak shape of acids.
Zorbax SB-C8	5/95 ACN/pH 1.5 H_3PO_4	Co-elution of the two acids
Platinum C8 EPS	5/95 ACN/pH 1.5 H_3PO_4	Poor separation of the two acids
Inertsil C4	5/95 ACN/pH 2.0 H_3PO_4	Co-elution of oxalic acid with solvent front
Kromasil C1	5/95 ACN/pH 2.0 H_3PO_4	Co-elution of the two acids
Aquasil C18	5/95 ACN/pH 2.0 H_3PO_4	Poor separation of the two acids
Hypercarb	0.05% TFA, pH 2.3	Poor separation and peak shape of acids
	5/95 ACN/pH 1.5 H_3PO_4	Same as above
Zorbax SB-CN	PH 7.0 H_3PO_4	Co-elution of all three analytes and the solvent front
	30/70 CAN/ 0.2% acetic acid	Same as above
	50/50 CAN/pH 7.0 H_3PO_4	Same as above
Discovery RP-amide C16	pH 7.0 H_3PO_4	Co-elution of oxalic acid, oxamic acid, and the solvent front
	15/85 MeOH/ pH 7.0 H_3PO_4	Same as above
	50/50 CAN/H_2O	Oxamide and solvent co-elute; no elution of the two acids
AquaSep C8	100% H_2O	Poor separation of the two acids
	pH 4.5 H_3PO_4	Same as above
	pH 2.5 H_3PO_4	Same as above

Table 3
Normal-phase HPLC screening conditions and results

Column	Mobile phase	Results
Zorbax CN	20% IPA/hexane	No elution of the two acids
	A, 20% IPA/ hexane[a]; B, 90% IPA/hexane	Poor separation and peak shape of the two acids; unstable baseline

[a] Gradient from 20/80 to 90/10 IPA/hexane over 30 min.

Table 4
Ion-exclusion HPLC screening conditions and results

Column	Mobile phase	Results
IonPac CS14	5/95 ACN/0.01 N HCl	Poor separation and peak shape of the acids
IonPac ICE-AS1	5/95 ACN/ 0.1% H_2SO_4	Good separation and peak shape of all analytes

As stated earlier, the IEC retention of organic acids depends primarily on the dissociation of the acid, with the strongest acid being the least retained [7,8]. Among the three oxalate-related compounds, oxalic acid has pK_a values of 1.23 and 4.19, oxamic acid has an estimated pK_a of 1.48 based on the acid-strengthening effects of substituents [16], and oxamide is a neutral compound. Therefore, it can be predicted that the three analytes would be eluted in the order of oxalic acid, oxamic acid and oxamide. The chromatograms in Fig. 2 are consistent with this prediction.

3.1.2. Optimization of IEC chromatographic conditions

Diluted mineral acids, such as HCl and H_2SO_4, are commonly used in IEC eluents. The mineral acids can suppress the ionization of weakly acidic analytes and therefore increase their retention. No significant difference in the separation of the oxalate-related compounds was observed using eluents with HCl or H_2SO_4. Considering the corrosive nature of HCl, dilute H_2SO_4 was selected for long-term use. Because ionization of organic acids depends on the pH, their elution behavior with IEC would be affected by changing the pH of the eluent. It has been demonstrated that, with IEC, the retention of weak acids would generally increase as the acid concentration increases or the pH of the eluent decreases [7,8,17]. The peak shape of the acids may also be improved with increased acid concentration [17]. In the present work, an eluent containing 0.1% H_2SO_4 was selected because it had given reasonable separation, peak shape and run time. The addition of an organic modifier to the eluent had been shown to reduce any hydrophobic interac-

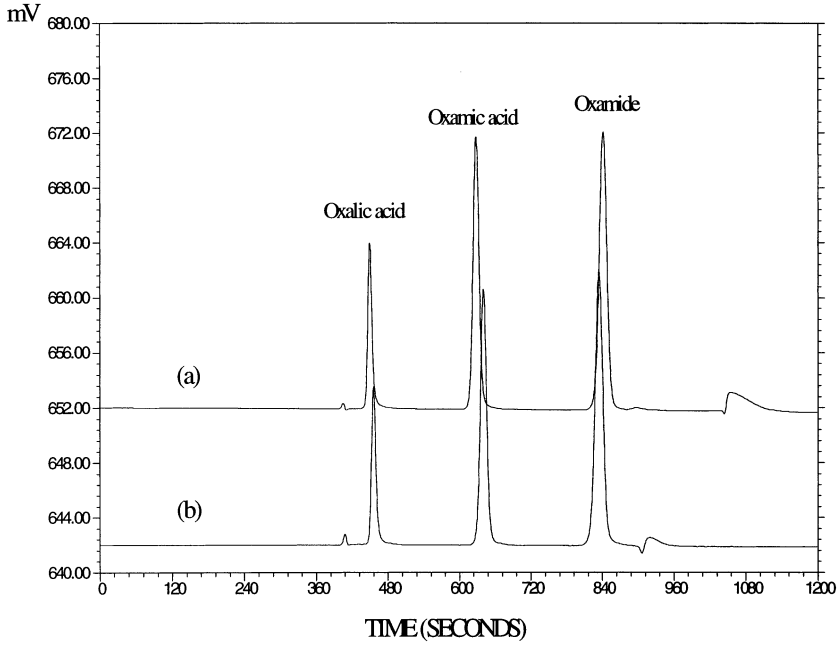
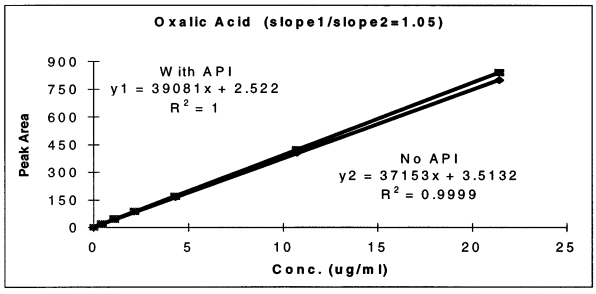
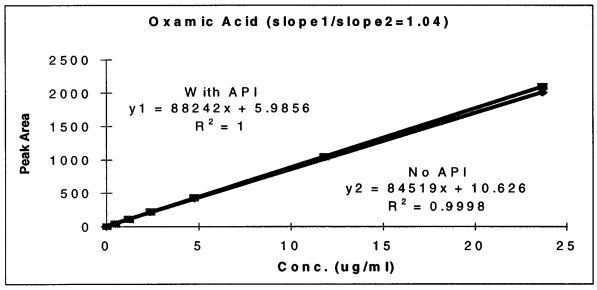


Fig. 2. Chromatograms using (a) 0.1% H_2SO_4 and (b) 5/95 (v/v) ACN/0.1% H_2SO_4 as eluent. Sample concentrations were 8.5, 8.1, and 4.3 μg/ml for oxalic acid, oxamic acid, and oxamide, respectively. Other conditions are the same as described in Section 2.





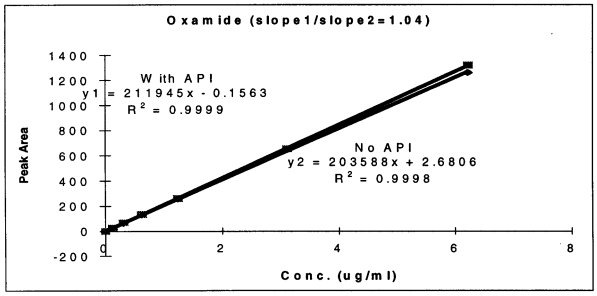


Fig. 3. Sample matrix effect and linearity test. Solutions of oxalic acid, oxamic acid and oxamide with no API were prepared in 30/70 ACN/H_2O; solutions with API were prepared in 30/70 ACN/H_2O containing 0.61 mg/ml API. All other conditions are the same as in Section 2.

tion between the organic analytes and the IEC resin and thus reduce the retention [7,18]. The present work showed that adding 5% ACN in the mobile phase slightly reduced the retention of oxamide and slightly improved the peak shape (Fig. 2). It was also found that the addition of 5% ACN helped to minimize any baseline disturbances caused by using a sample solvent containing an organic component. However, regular use of higher ACN content in the eluent is not recommended for IEC columns. Therefore, only 5% ACN was used in the eluent for the present work.

In order to obtain adequate solubility of a hydrophobic API sample matrix, ACN/water, 30/70 (v/v) was used as the sample solvent. Higher ACN content in the sample solvent, however, may result in baseline disturbances due to the very low ACN content of the eluent.

Oxalic acid, oxamic acid, and oxamide are all poorly UV-absorbing compounds; therefore, a low wavelength, 205 nm, was used for detection.

3.2. Method validation

3.2.1. Matrix effect

The IEC method with the optimal conditions was applied to the determination of low-level oxalic acid, oxamic acid, and oxamide in a hydrophobic API matrix (proprietary structure). The API did not elute after numerous injections under the experimental conditions, indicating that it was strongly retained on the column. A possible matrix effect was assessed by analyzing solutions spiked with oxalic acid, oxamic acid, and oxamide, with and without the presence of the API. No change in resolution or peak shape of the three oxalate-related compounds was observed with the presence of the API matrix. Also, the linearity plots obtained from the samples with the API were compared to those obtained from standard solutions without the API as shown in Fig. 3. The linear regression slope with the API-containing samples was within 5% of the slope of the standard solutions for all three oxalate-related analytes, indicating the absence of a significant matrix effect with the IEC method.

To determine the potential impact of the accumulation of the API matrix on the column, 60 repeated injections of the API sample solution were made on two separate ICE-AS1 columns, and no significant change in separation was observed after the repeated injections on either column. The accumulation of the API matrix on the column can be washed out by rinsing the column with 50/50 (v/v) ACN/0.1% H_2SO_4 at 0.8 ml/min for 30 min after each run. However, 50/50 (v/v) ACN/0.1% H_2SO_4 can only be used for a brief rinse; a 0.1% H_2SO_4 solution is recom-

mended for column storage. Using a guard column or disk to retain the API matrix before it entered the IEC analytical column was not successful due to the low capacity of the guard column or disk.

3.2.2. Linearity

Linearity was evaluated using six standards in the concentration range 0.4–21.4 μg/ml for oxalic acid, 0.5–23.7 μg/ml for oxamic acid, and 0.1–6.2 μg/ml for oxamide. As shown in Fig. 3, the responses of all three analytes are linear over the concentration ranges tested.

3.2.3. Limit of quantitation (LOQ)

The LOQ was estimated using the following equation as recommended by the ICH guideline for method validation [19]

$$\mathrm{LOQ} = \frac{10\sigma}{S}$$

where σ was the residual standard deviation from the linear regression of the standard curve, and S was the slope of the regression line.

The estimated LOQ values are 0.4 μg/ml for oxalic acid, 0.6 μg/ml for oxamic acid, and 0.2 μg/ml for oxamide, which are equivalent to 0.08, 0.12, and 0.04%, respectively, in an API sample solution of 0.5 mg/ml.

3.2.4. Precision

The method demonstrated adequate precision as evaluated by assaying two sets of six replicate sample preparations at two concentration levels. The relative standard deviations of the normalized responses were 9.3% at $\sim$1.0 μg/ml and 12.4% at $\sim$0.4 μg/ml for oxalic acid, 4.1% at $\sim$1.0 μg/ml and 3.8% at $\sim$0.4 μg/ml for oxamic acid, and 3.4% at $\sim$0.6 μg/ml and 3.7% at $\sim$0.2 μg/ml for oxamide. The lower concentration levels for each oxalate-related compound are close to the estimated LOQ values for these compounds.

3.2.5. Robustness

The robustness of the IEC method was examined by varying the column temperature, the concentration of H_2SO_4, and the ACN content in the eluent around the nominal conditions. The retention of oxalic and oxamic acids increased slightly with increased concentration of H_2SO_4 from 0.08 to 0.12% as can be predicted [7,8,17]. The retention of oxamic acid and oxamide decreased slightly with increased ACN content from 3 to 7% due to reduced hydrophobic interaction between the analytes and the IEC resin [7,18]. The retention of oxamic acid and oxamide also decreased slightly with increased column temperature from 18 to 40°C. No change in retention was observed with oxalic acid upon temperature change, which is consistent with an earlier observation in the literature [8]. In all cases, no significant changes in resolution or peak shape were observed.

The IEC method was also tested in two different laboratories and with both new and aged columns. The separation profiles of the three oxalate-related compounds obtained from the two laboratories and with the different columns were comparable.

All three analytes, both with and without the API, showed $< 10\%$ change in response after 24 h storage under ambient conditions.

4. Conclusions

Reversed-phase and normal-phase methods with various columns have generally shown poor separation of oxalic acid, oxamic acid, and oxamide. An ion-exclusion chromatography method using a Dionex IonPac ICE-AS1 column demonstrated good separation and LOQ for oxalic acid, oxamic acid, and oxamide. The method has been successfully validated and applied to the determination of these oxalate-related impurities at low levels in an API matrix.

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