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Short communication

Separation of rutin nona(H-) and deca(H-) sulfonate sodium by ion-pairing reversed-phase liquid chromatography

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Abstract

Ion-pairing reversed-phase liquid chromatography (RPLC) was used to separate two polysulfonates, rutin nona(H-) sulfonate sodium and rutin deca(H-) sulfonate sodium, which have very similar chemical structures. The final product always contained both of them when one of the compounds was synthesized. Baseline separation was achieved on a C_8-bonded silica column at ambient temperature. The eluent was acetonitrile–15 mM phosphate buffer solution containing 20 mM TBA (pH 6.0) (46:54, v/v). The calibration plot was linear in the concentration range 0.5–200 μg ml^{-1} for both analytes. The limits of detection (LODs; 254 nm) were 0.03 μg ml^{-1} for rutin nona(H-) sulfonate sodium and 0.04 μg ml^{-1} for rutin deca(H-) sulfonate sodium. Three batches of rutin deca(H-) sulfonate sodium were analyzed using the assay; the results showed that the analytical performance is really satisfactory.

Keywords: Rutin nona(H-) sulfonate sodium; Rutin deca(H-) sulfonate sodium; Flavones, polysubstituted

1. Introduction

Ion chromatography is a common and widely used technique for the separation of ionic compounds. Two types of ion chromatography are in practical use; that are based on either an ion-exchange mechanism or an ion-pairing formation and hydrophobic interaction mechanism. A variety of approaches [1–7] have been used for the resolution of polysulfonated compounds by ion-pairing reversed-phase liquid chromatography (RPLC), but no studies on the resolution of compounds with more than four sulphonic substituents have been reported. In this study, rutin nona(H-) sulfonate sodium and rutin deca(H-) sulfonate sodium, polysubstituted flavones, were separated on the base of ion-pair formation. The two analytes have almost the same chemical structure; the sole difference in their structure is one SO_3Na group in the C5-OH portion (Fig. 1). As far as we are aware, there is no published method for the separation of rutin nona(H-) and deca(H-) sulfonate sodium. In this study, ion-pairing RPLC with tetrabutyl ammonium bromide (TBA) as ion-pairing reagent in the LC eluent was used to separate the target compounds.

2. Materials and methods

2.1. Reagents

Rutin nona(H-) sulfonate sodium and rutin deca(H-) sulfonate sodium were kindly donated by Dr. Yong-Zhou Hu (Department of Medicinal Chemistry, Zhejiang University, China). Tetrabutyl ammonium bromide (analytical grade) was purchased from Wu-lian Chemical Factory (Shanghai, China). Acetonitrile was of HPLC-grade and obtained from Tedia Company (Fairfield, Texas, USA). Monopotassium phosphate and sodium hydroxide were from Huzhou Chemical Factory (Huzhou, China). All other chemicals and solvents were of analytical-reagent or HPLC-grade.

2.2. Apparatus

The LC system consisted of an LC-10ATvp pump (Shimadzu, Kyoto, Japan) equipped with a Rheodyne injector with a volume loop of 20 μl and an SPD-10Avp

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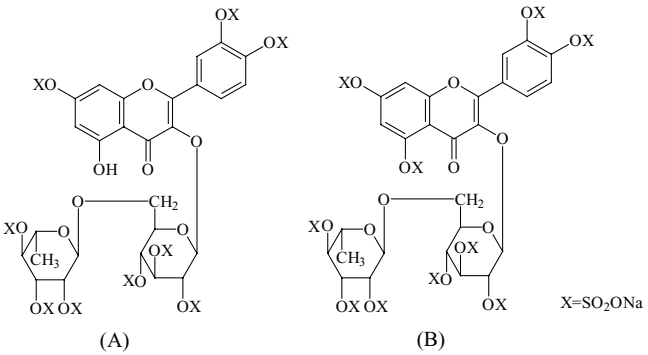

Fig. 1. Chemical structures of rutin nona(H-) and rutin deca(H-) sulfonate sodium.

UV-Vis detector and connected with a HS2000 Chemstation (Hangzhou, China). The analytical column was packed with C_8-bonded silica (150 mm × 4.6 mm i.d.; particle size 5 μm) (Agilent, Palo Alto, CA, USA).

2.3. Procedure

The eluent was acetonitrile–15 mM phosphate buffer solution containing 20 mM TBA (pH 6.0) (46:54, v/v). The buffer solution was adjusted to various pH values measured with a Mettler 320 pH meter, by adding a few drops of sodium hydroxide solution (1 M). The eluent was filtered through a 0.45 μm membrane. All eluents were freshly prepared daily. The flow-rate was 1.0 ml min^{-1} and the detection wavelength was 254 nm (output range: 0.010 AUFS). The analysis was carried out at ambient temperature. The mixture sample of rutin nona(H-) sulfonate sodium and rutin deca(H-) sulfonate sodium was dissolved in eluent to give concentration of about 100 μg ml^{-1}. Injections were made when the baseline was stable.

3. Results and discussions

3.1. Ion-pairing reagent

The concentration of an ion-pairing reagent in the eluent is an important parameter for the separation of mono- or di-sulphonated compounds by RPLC [8,9]. In this paper, the separation of the two analytes was studied after adding TBA to the buffer solution in 5–25 mM concentrations. An increase in the concentration from 15 to 25 mM resulted in shorter retention times of rutin nona(H-) and rutin deca(H-) sulfonate sodium. No retention and separation of the two analytes was observed when the concentrations were less than 10 mM. Probably, the lower concentrations of TBA do not provide enough ion-pairing groups for the large number of sulfonate sites. On the other hand, the separation did not improve at concentrations higher than 25 mM. Table 1 shows relevant retention time, plate number and resolution data. As is obvious, the best results, especially requiring peak shape, were observed at 20 mM TBA.

Sulfonates are eluted close to the column dead volume with little separation and often even with strongly asymmetric peak shapes in reversed-phase systems when pure aqueous-organic eluent were used [10]. In the case of polysulfonated compounds, if the concentration of the ion-pairing agent is sufficiently high to pair all sulfonate groups, monosulfonates will elute prior to di- and trisulfonates. At low concentration, however, only one sulfonate group per molecule will be paired, and the elution order is inverted [11]. In order to increase the retention and achieve a successful separation, it is necessary to have sufficient ion-pairing agent in the mobile phase.

3.2. pH value

The effect of pH was studied by using a fixed concentration (20 mM) of TBA in the buffer solution and carefully equilibrating both the resin and the eluent at the pH values. Typical results are given in Table 2. An increasing pH caused the retention time to become shorter. The best conditions in terms of retention, peak shape and resolution for the two analytes were obtained at pH 5–7, with an optimum at pH 6.0.

The eluent pH [12,13] was frequently investigated and is recommended to be above the pK_a values of the analytes to ensure dissociation of the acidic groups and strong ion-pairing formation [13]. pH values typically vary from slightly acidic (pH 5) [13] to neutral [14]. For stable conditions the pH should be in a range where small changes in

Table 1
Effect of TBA concentration on retention time, t_R, plate numbers, N, and resolution, R, of the test analytes

Analyte	TBA concentration (mM)				
	5	10	15	20	25
Deca(H-) sulfonate					
t_R (min)	No retention	No retention	17.2	9.9	8.3
N			2100	3700	3200
Nona(H-) sulfonate					
t_R (min)	No retention	No retention	21.0	12.1	10.1
N			2400	2600	2300
Resolution, R	–	–	2.7	2.8	2.7

Table 2
Effect of pH on retention time, plate numbers and resolution of the test analytes

Analyte	pH				
	4.4	5.2	6.0	6.8	7.4
Deca(H-) sulfonate					
t_R (min)	16.8	10.1	8.6	7.7	5.8
N	2800	3300	3700	3200	2800
Nona(H-) sulfonate					
t_R (min)	19.5	12.1	10.2	9.2	6.8
N	1700	2100	2500	2400	2400
Resolution, R	1.7	2.2	2.4	2.3	2.3

pH have only a limited effect on the analyte retention. In the present case, this is true for pH values of about 6.

3.3. Salt concentration in buffer solution

In addition to the TBA concentration and the pH, the electrolyte concentration (inorganic modifier) will also influence retention. Furthermore, at high TBA and electrolyte concentrations in the buffer solution, the LC column can easily be damaged. That is, a suitable compromise requiring separation and column protection has to be made. The data of Table 3 show that the presence of 15 mM monopotassium phosphate in the buffer solution is an appropriate choice.

The retention of the two analytes decreases with increasing monopotassium phosphate concentration, since the inorganic anions complete with the analytes in the ion-pairing formation. The aspect has to be considered if aqueous samples with high salt contents are analyzed directly.

Under the optimum conditions, the separation was fully satisfactory, as becomes clear from Fig. 2.

3.4. Analytical performance

The intra and inter-day precision were obtained by analyzing spiked samples containing rutin nona(H-) and deca(H-) sulfonate sodium at three concentrations (5.0, 20 and 100 $\mu g\,ml^{-1}$) in five replicates within one day and on five consecutive days, respectively (Table 4).

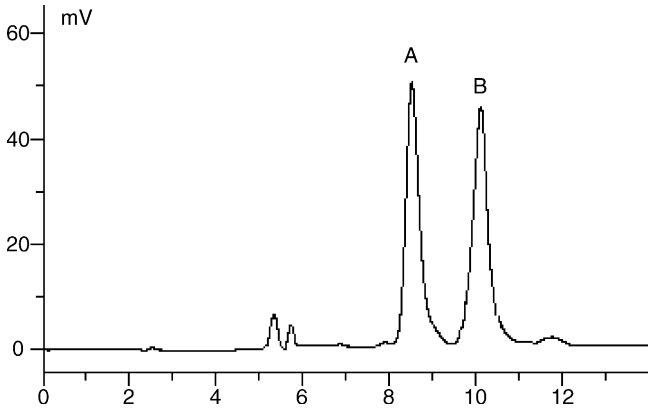

Fig. 2. Ion-pairing RPLC of rutin nona(H-) and rutin deca(H-) sulfate sodium; UV detection at 254 nm.

The peak area (Y) versus concentration (X) calibration curves for the two analytes were linear over the concentration range 0.5–200 $\mu g\,ml^{-1}$, i.e. rutin nona(H-) sulfonate sodium, $Y = 12183.2X - 3014.7$, $r = 0.9999$; rutin deca(H-) sulfonate sodium, $Y = 9218.7X - 4149.3$, $r = 0.9998$.

The limits of detection (LODs), defined as the lowest concentration which can be detected (signal-to-noise

Table 3
Effect of monopotassium phosphate concentration on retention time, plate numbers and resolution of the test analytes

Analyte	Concentration (mM)			
	5	10	15	20
Deca(H-) sulfonate				
t_R (min)	47	17.1	9.9	7.6
N	3100	3100	3200	3100
Nona(H-) sulfonate				
t_R (min)	54	19.8	12.0	9.7
N	2600	2900	2900	2900
Resolution, R	1.8	2.5	2.7	2.6

Table 4
Intra-day and inter-day precision (R.S.D.) of the proposed method ($n = 5$)

Target concentration ($\mu g\,ml^{-1}$)	Nona(H-) sulfonate sodium		Deca(H-) sulfonate sodium	
	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)
5.0	2.0	2.8	1.8	2.2
20	1.7	2.1	1.0	2.0
100	1.9	2.6	1.1	1.8

ratio 3) were $0.03\,\mu g\,ml^{-1}$ for rutin nona(H-) sulfonate sodium and $0.04\,\mu g\,ml^{-1}$ for rutin deca(H-) sulfonate sodium.

3.5. Application

The proposed method was used to determine the contents of the two analytes in three samples of rutin deca(H-) sulfonate sodium synthesized by the Department of Medicinal Chemistry of the Zhejiang University. As mentioned before, the final product will always contain both compounds irrespective of which one is synthesized. That is, in this case each batch of rutin deca(H-) sulfonate sodium contained a small amount of rutin nona(H-) sulfonate sodium. The content of deca(H-) and nona(H-) sulfonate were 94.4–98.7% and 5.1–1.0%, respectively.

4. Conclusion

Ion-pairing RPLC with TBA as reagent gives baseline separation of rutin nona(H-) sulfonate sodium and rutin deca(H-) sulfonate sodium with satisfactory UV-Vis detection at 254 nm. The method can be used to determine the target compounds in real-life mixtures.

Acknowledgements

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