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Short communication

Simultaneous determination of oxalic, fumaric, maleic and succinic acids in tartaric and malic acids for pharmaceutical use by ion-suppression reversed-phase high performance liquid chromatography

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Abstract

A reliable method for the simultaneous determination of oxalic, fumaric, maleic, and succinic acids in tartaric and malic acids for pharmaceutical use by reversed-phase ion-suppression high performance liquid chromatography is presented. HPLC was achieved on a Nova-Pak C_{18} column by isocratic elution using water adjusted to pH 2.10–2.15 with perchloric acid, and detection was by UV adsorption at a wavelength of 210 nm. This method was found to be superior to previous liquid chromatography as well as other classical assay, and to be an attractive choice for the analysis of these compounds. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Tartaric acid; Malic acids; Oxalic acid; Fumaric acid; Maleic acid; Succinic acid; Ion-exchange chromatography; HPLC; Perchloric acid

1. Introduction

Tartaric acid and malic acid, which have similar action, are commonly used in effervescent saline pharmaceutical preparations for desloughing of wounds and ulcers, etc. They are permitted food additives as well [1,2]. As impurities, oxalic, fumaric, and maleic acids are officially limited in tartaric and malic acids. The statutory methods for the determination of oxalic acid in tartaric acid are turbidimetric and colorimetric methods, and that for the determination of fumaric and maleic acids in malic acid is ion-exchange chromatography [3,4]. The allowable content of succinic acid in tartaric or malic acid has not been officially defined so far. Turbidimetry by means of calcium oxalate precipitation and colorimetry by

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means of zinc ferricyanide color development are time-consuming and only achieve limited rather than exact results. The disadvantages of ion-exchange chromatography are the use of a strongly acidic cation exchanger which has a lower exchange capacity, the long time required for equilibrium, separation and re-equilibrium. Reversed-phase high performance liquid chromatography (HPLC) has become more and more popular for analyzing certain mixtures of organic acids because of the simplicity, rapidity and stability of the method, but most of the previous studies with regard to the determination of carboxylic acids are in fruits including their products such as apple juice, cider, must and wine, as well as plant extracts and tissues, etc. [5–10]. In this paper a reversed-phase ion-suppression HPLC method is presented in which oxalic, fumaric, maleic and succinic acids in tartaric and malic acids can be determined simultaneously for pharmaceutical purposes.

2. Experimental

2.1. Apparatus and chromatographic conditions

The chromatographic analysis was performed with a Waters 510 solvent pump (Waters, Mildford, MA), a Rheodyne 7725i injector valve equipped with a 20 μl loop (Rheodyne, Cotati, USA), and a Waters 486 tuneable UV absorbance detector (Waters). The chromatograms were recorded on a Yokogawa Hokushin electric type 3066 pen recorder (Sino-Japanese Sichuan Fourth Meter Factory, Chongqing, PRC), and a model JS3030 chromatographic working station (Dalian Jiangshen Separation Science and Technology, Dalian, PRC).

The column used was a Nova-Pak C_{18}, 150 mm × 3.9 mm i.d. stainless steel analytical column with 4 μm particle size (Waters). The mobile phase was water adjusted to a pH between 2.10 and 2.15 with perchloric acid and filtered through a cellulose membrane with 0.45 μm micropores (Millipore, Belford, MA). The separation was carried out by isocratic elution with a flow rate of 1.0 ml min^{-1} and the column temperature was maintained constant at 30°C. The UV detector was set at 210 nm with a sensitivity of 0.02 absorbance units, full scale. Quantitation was based on the peak area measurement.

2.2. Reagents and chemicals

Water from a Millipore Milli-Q system was used for all solutions, dilution and the mobile phase. Perchloric acid (70–72%) obtained from Shanghai Taopu Chemical Plant (Shanghai, PRC) was a guaranteed reagent. Analytical grade organic acids without further purification were used as standards: tartaric, malic, oxalic, fumaric, maleic, and succinic acids were purchased from Aldrich (Milwaukee, WI), E. Merck (Rahway, NJ), Tianjing Second Reagent Factory (Tianjing, PRC), Shanghai First Reagent Factory (Shanghai, PRC), and Nanjing Chemical Reagent Factory (Nanjing, PRC). Tartaric and malic acids samples were provided by Nanjing Pharmaceutical Plant (Nanjing, PRC) and Sinochem Jiangsu (Nanjing, PRC).

A mixture of all the acids studied was used to optimize the peak resolution. The standard of the individual acid was prepared in the mobile phase and chromatographed separately in order to determine the retention time for each acid. Calibration mixtures made of oxalic, fumaric, maleic and succinic acids were used. The standard solutions used for calibration purpose were prepared by dissolving the appropriate amount of each carboxylic acid in the mobile phase, and diluting to a final concentration of 0.005–0.05 mg ml^{-1} for oxalic and succinic acids and 0.0005–0.005 mg ml^{-1} for maleic and fumaric acids. The sample solutions were prepared singly by weighing out 0.1 g tartaric or malic acid for pharmaceutical use into a 100-ml volumetric flask and adding the mobile phase to make up to the mark. All solutions were filtered through a Millipore filter (0.45 μm) by means of a 10-ml syringe before injection.

3. Results and discussion

An HPLC chromatogram demonstrating the separation of a mixture of the acids studied is

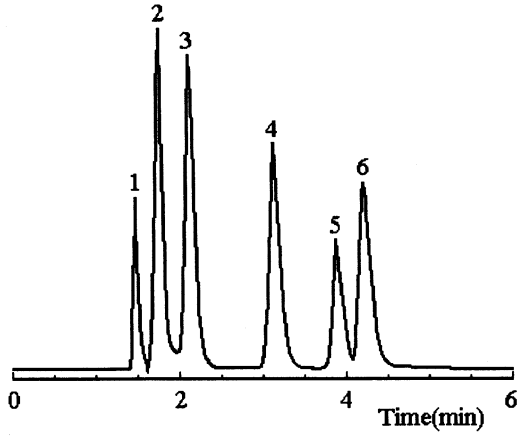

Fig. 1. Chromatogram of a standard organic acid mixture. The acids are: 1, oxalic; 2, tartaric; 3, malic; 4, maleic; 5, succinic; and 6, fumaric.

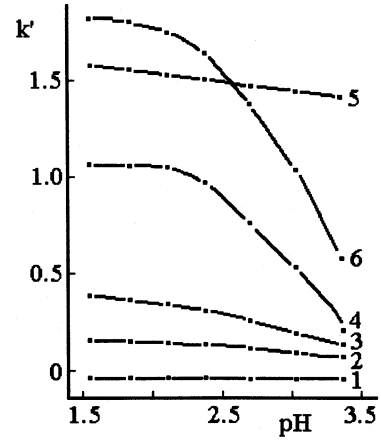

Fig. 2. Effect of the mobile phase pH upon the capacity factor of organic acids. The acids are as in Fig. 1.

shown in Fig. 1. As can be seen the order of the retention time is oxalic, tartaric, malic, maleic, succinic, and fumaric acid with a relative retention time of 0.71, 0.84, 1.00, 1.52, 1.88, and 2.04 min, respectively, and the comprehensive resolution among the acids is improved. Ion-exchange chromatography gave a relative retention time of approximately 0.6 for maleic, 1.0 for malic acid, and about 1.5 min for fumaric acid [3]. Table 1 shows that the capacity factor k' for the constituents was consistent with the first acid ionization constant pK_{a1} except for maleic and fumaric acid which contain a carbon-–carbon double bond, and is also consistent with the octanol–water partition coefficient log P_{oct} except for oxalic acid which has too small a pK_{a1} value. Oxalic acid appears before the eluent not only because of its lack of hydrophobic groups suggested by a previous author [9] but also due to its dissociation at pH 2.10–2.15.

Table 1
Capacity factors, first acid ionization constants and octanol–water partition coefficients for carboxylic acids

Acid	k'	pK_{a1} [11]	Log P_{oct} [12]
Oxalic	−0.04	1.23	−0.7
Tartaric	0.14	3.22	−1.8
Malic	0.34	3.40	−1.4
Maleic	1.05	1.83	−0.7
Succinic	1.52	4.16	−0.6
Fumaric	1.74	3.03	0.2

Phosphoric and sulfuric acid are the most commonly used as ion-suppressants for the determination of organic acids on the C_{18} column by reversed-phase HPLC [5–9], however, perchloric acid has been successfully employed because of its stronger acidity and suppressive action at extremely low application concentration [13,14]. Only several drops of reagent grade perchloric acid makes 1 l of water with a pH of approximately 2. Using this mobile phase, the desired acids were clearly separated. It was found that the suppressive action of 0.0018 mol l^{-1} aqueous perchloric acid (pH 2.10–2.15) was not less than that of 0.0074 mol l^{-1} aqueous phosphoric acid, or 0.01 mol l^{-1} potassium dihydrogen phosphate adjusted to pH 2.25 with phosphoric acid, or aqueous sulfuric acid at pH 2.45–2.50 with an adjusted ionic strength of 0.10 mol l^{-1} with disodium sulfate. Furthermore, the perchloric acid concentration is so low that the total analysis time is shortened because the chromatographic system is easy to equilibrate, and the column life is increased because the column is easy to rinse after analysis.

The mobile phase was adjusted to different pHs with perchloric acid in order to select a suitable acidity. The observed influence of pH on the total retention time of the six acids studied is shown in

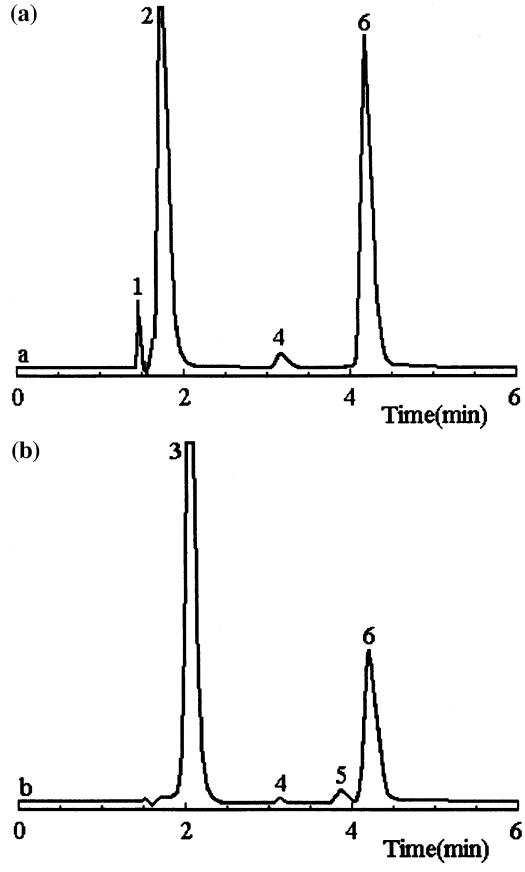

Fig. 3. Chromatograms of pharmaceutical-use samples. (a) Tartaric acid; (b) malic acid. The acids are as in Fig. 1.

Fig. 2, parts of which basically agree with the previous results obtained from a reversed-phase C_{18} column by using other acids or buffers in the mobile phase as suppressants [5,9].

A linearity has been obtained for each acid inside the range studied with a correlation coefficient higher than 0.999 except for oxalic acid (0.998). The lowest detectable concentration in this system is less than 0.0005 mg ml^{-1} for oxalic and succinic acid, 0.00002 mg ml^{-1} for maleic acid, and 0.00001 mg ml^{-1} for fumaric acid. Typical chromatograms for these acids in pharmaceutical-use tartaric and malic acid are shown in Fig. 3. In the present study, four random samples of industrial or commercial products were analyzed for carboxylic acid impurity content. The result is summarized in Table 2. It can be seen that the precision of oxalic acid analysis is significantly less than that of the other acid because it emerges so early that it is interfered with by the solvent. If the mobile phase is not strictly pure, the deviation could be serious.

4. Conclusions

It was found that the ion-suppression reversed-phase HPLC technique described in this paper has two significant features: (1) The use of extremely dilute perchloric acid in water as the mobile phase decreases the total analysis time and increases the column life. (2) Good separation of the trace and major constituents in real samples facilitates simultaneous determination of all the acids of interest. The procedure make it a convenient and economical alternative to earlier methods for determining oxalic, fumaric, maleic and succinic acids in tartaric and malic acids for pharmaceutical use.

Table 2
Precision of the proposed method

Sample	Batch No.	Oxalic acid		Maleic acid		Succinic acid		Fumaric acid	
		Content	R.S.D.	Content	R.S.D.	Content	R.S.D.	Content	R.S.D.
Tartaric acid	960311	0.028	6.4	0.0082	3.6	ND	—	0.70	3.1
	960408	0.025	7.0	0.0072	3.2	ND	—	0.69	2.8
Malic acid	960107	ND	—	0.0016	4.1	0.41	3.0	0.11	3.8
	960423	ND	—	0.0018	3.8	0.45	3.0	0.15	3.5

Values are in percent, $n = 6$.

Acknowledgements

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