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Sodium benzoate and potassium sorbate preservatives in food stuffs in Iran

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A high-performance liquid chromatography method was applied for the determination of the levels of benzoate and sorbate in 400 food samples, including pickled cucumbers, canned tomato pastes, sour cherry jams, soft drinks, fruit juices and dairy products (UF-Feta cheeses, Lighvan cheeses, lactic cheeses, yogurts and doogh). The results showed that 270 (67.5%) of all samples contained benzoate ranging from 11.9 to 288.5 mg kg^{-1} in lactic cheese and fruit juice, respectively. The levels of sorbate in 98 (24.5%) of the samples were 20.1 to 284.3 mg kg^{-1} in doogh and fruit juice, respectively. Moreover, benzoate was detected in all dairy products ranging from 11.9 mg kg^{-1} in lactic cheese to 91.2 mg kg^{-1} in UF-Feta cheese. A low concentration of benzoate could originate naturally, due to specific biochemical mechanisms during cheese, yogurt and doogh maturation. In conclusion, a minimum level for benzoate in dairy products should be defined in the legislation.

Keywords: food; cheese; sodium benzoate; potassium sorbate; HPLC

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Introduction

Microbiological, enzymatic or chemical changes can occur during shelf life of food because of their high levels of nutrients, thus preservatives are added to stop or delay nutritional losses. The use of preservatives such as benzoate and sorbate has become more important to control microbial growth and extend the shelf life of foods (El-Ziney 2009). Benzoic and sorbic acid and their respective sodium and potassium salts are commonly used preservatives for food protection (Akbari-adergani et al. 2013).

Sorbates and benzoates are permitted in some foods in Europe (European Commission 2008). However, the use of these preservatives is not allowed in some foods such as dairy products, fruit juice and canned tomato paste, by the Institute of Standard and Industrial Research of Iran (ISIRI) and its presence must be declared on the label (Mota et al. 2003; ISIRI: No. 2453 2008; ISIRI: No. 761 2012; ISIRI: No. 14345 2012).

In the code of federal regulation under the provision set forth by the FDA (US Food and Drug Administration) on potassium sorbate in 1999 is declared that if food additives are generally recognised as safe (GRAS) and declared on the label, they can be used in foods. Moreover, benzoic acid and its salt were evaluated several times by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1973), and were found to be acceptable for use in foods. However, benzoate may cause allergic reactions in sensitive individuals as well as hyperactivity, especially when used with food colourants (Bateman et al. 2004). Sorbic acid was also reported to be a nucleophilic compound and has low toxicity because it is rapidly metabolised by pathways similar to fatty acids (El-Ziney 2009).

The determination of these preservatives is not only important for quality assurance purposes, but also for human safety. There are various methods for analyses of benzoates and sorbates in food products, but the most common method in the food industry (Saad et al. 2005) is reversed-phase high-performance liquid chromatography (HPLC).

The aim of this study is to determine benzoate and sorbate levels in seven food types, including pickled cucumber, canned tomato paste, sour cherry jam, carbonated soft drink, fruit juice and dairy products (ultrafiltrated feta cheese (UF-Feta cheese (Iranian cheese)), Lighvan cheese (Iranian cheese), lactic cheese (made by denaturation of milk proteins by adding yogurt to milk), yogurt and doogh (Iranian dairy drink) by HPLC.

Methods and materials

Chemicals and reagents

Potassium sorbate (>99%), sodium benzoate (>99%), sodium salicylate (>99.5%), ammonium acetate (98%), glacial acetic acid (100%) and HPLC-grade acetonitrile used in this study were obtained from Merck (Darmstadt, Germany). Deionised water was prepared by a Thermo Scientific Bran stead Easy pure II system (Thermo Fisher Scientific Inc. Waltham, MA, USA).

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Sampling

In this study, 400 food samples classified into seven categories were purchased from supermarkets located in Sari. These categories were pickled cucumber, canned tomato paste, sour cherry jam, carbonated soft drink, fruit juice and dairy products (ultrafiltrated feta cheese (UF-Feta cheese (Iranian cheese)), Lighvan cheese (Iranian cheese), lactic cheese, yogurt and doogh (Iranian dairy drink). For each type, four brands were chosen and at least 10 samples from each brand were purchased. After samples were analysed, their averages were reported in this study.

Chromatographic conditions

Chromatographic analysis was carried out on an Agilent 1200 series HPLC system (Agilent Technologies, Santa Clara, CA, USA), equipped with a 20 µL loop injector, UV Detector and an Eclipse-XDB C_{18} column (150 × 4.6 mm, 5 µm). The mobile phase consisted of an aqueous ammonium acetate buffer (pH = 4.2) and acetonitrile (80:20 v/v) with a flow rate of 0.8 mL min^{-1}. The injection volume was 20 µL and the UV detector was set at 225 nm.

Preparation of standards and samples

Individual standard stock solutions of sodium benzoate and potassium sorbate were prepared in distilled water (1000 mg L^{-1}). Working standard solutions of each preservative were prepared by diluting the stock solutions appropriately with distilled water to give a concentration between 31. mg L^{-1} and 500 mg L^{-1}. Mixed standard solutions, which contained two preservatives at concentrations between 3.1 mg L^{-1} and 50 mg L^{-1} with 20 mg L^{-1} of sodium salicylate as the internal standard, were also prepared by mixing and appropriately diluting the working solutions.

For preparation of samples, a volume of 10 mL or g was diluted with deionised water to 50 mL in a volumetric flask and shaken for 2 min. Then 2.0 mL of each diluted sample was mixed with 200 µL of the internal standard (1000 mg L^{-1}), diluted by mobile phase to 10 mL and shaken vigorously for 2 min. The samples were then filtered through a 0.45 µm cellulose acetate syringe filter (Schleicher & Schuell, Dassel, Germany) and a volume of 20 µL was injected into the column.

Method validation

The method was validated by international guidelines (International Conference on Harmonization (ICH)), as described by Walfish (2006). The calibration curves of each salt were constructed over a range of 3.125–50 mg L^{-1}. Linearity between the concentration of both benzoate and sorbate salt and the peak areas was obtained and the correlation coefficient for each standard curve was calculated. The limit of detection (LOD) and quantification (LOQ) were determined by considering, respectively, 3.3 and 10 times the residual standard deviation of a regression line to the slope of ;the calibration curve (Walfish 2006). In order to verify the feasibility of the method, sample recovery was used by analysing samples before and after the addition of known quantities of benzoate and sorbate. In addition, to evaluate the intraday precision (as RSD_r), each sample was extracted three times and each sample extract was analysed on the same day. Quality control was performed by repeated analysis of a certified reference material (CRM T2077, a soft drink, from FAPAS, the Food Analysis Performance Assessment Scheme, the Food and Environment Research Agency, Sand Hutton, York, UK), which is also applied to estimate measurement uncertainty (Table 1). The CRM was evaluated for three times in 3 days. Thereafter, twice the standard deviation was reported as measurement uncertainty at a confidence level of 95%.

Table 1. Characteristics of CRM T2077, a soft drink from FAPAS.

Preservative	Assigned value (mg kg^{-1})	Satisfactory range (mg kg^{-1})	Measured (mg kg^{-1})
Benzoic acid	341	296–389	341
Sorbic acid	280	242–319	277

Statistical analysis

The acquired data were analysed using the SPSS statistical package, version 16 (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was utilised to evaluate the differences of distribution between the 13 brands. The results were expressed as mean ± SD for 10 samples of triplicate analyses in all tables. Statistical significance was set at $p < 0.05$.

Results and discussion

A chromatogram of the mixture of salicylate, benzoate and sorbate, with retention times 3.1, 7.4 and 9.3 min, respectively, is given in Figure 1. Peak identification was based on comparing the retention times of standard compounds and quantification was achieved by the external standard method using calibration curves fitted by linear regression analysis. Under optimised experimental conditions good linearity was observed between peak area response and concentration in the range 3.1–50 mg kg^{-1}. The results of

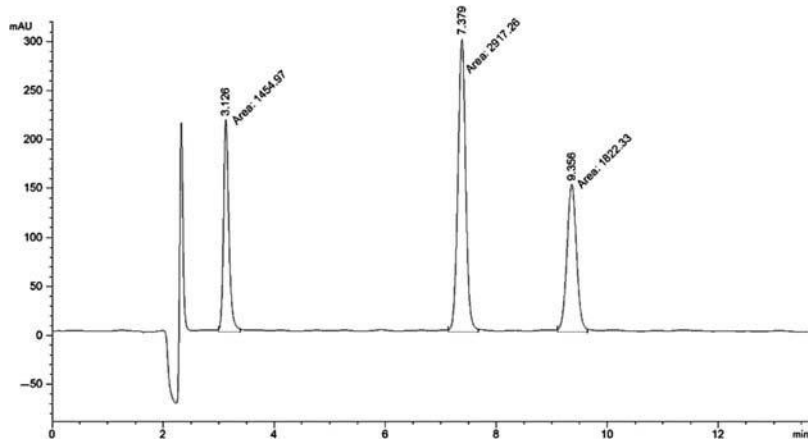

Figure 1. HPLC chromatogram of standard solutions (50 mg kg^{-1}) of (1) sodium salicylate, (2) sodium benzoate and (3) potassium sorbate.

Table 2. Analytical characteristics of the method validation.

Analyte	Calibration equation	R^2	Linear range (mg kg^{-1})	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	RSD_r % (n = 3)	Measurement uncertainty (mg kg^{-1})	Accreditation
Sodium benzoate	$y = 121.3x - 56.8$	0.9	3.1–50.00	0.6	1.9	0.9	±3.3	yes
Potassium sorbate	$y = 69.6x + 36.6$	0.9	3.1–50.00	0.5	1.5	1.0	±3.7	yes

calibration data, LOD, LOQ and recovery are presented in Tables 2 and 3. Recoveries ranged from 96.0% for potassium sorbate in pickled cucumber to 102.1% for sodium benzoate in yogurt indicating acceptable accuracy and suitability for simultaneous determination of these preservatives in various types of food.

Table 4 presents benzoate and sorbate levels as determined in the investigated samples. The occurrence of these two preservatives shows that a total of 75% of the samples were positive for at least one of these preservatives. The most prevalent preservative was benzoate with 67.5% of the total samples testing positive at mean values of 46.7 ± 43.0 mg kg^{-1}. In addition, 24.5% of samples were positive for sorbate at mean values of 79.2 ± 59.7 mg kg^{-1}. As shown in Table 4, both preservatives were not detected in any pickled cucumber samples. To our knowledge, there is one report of the determination of benzoate in pickled cucumber with UV-VIS spectrophotometer (Delavar et al. 2012). They reported that the benzoate level was near zero in canned pickles and pickled cucumbers from producing factories, but its level in bulk cargo pickled cucumber products was above the permissible level (200–400 mg kg^{-1}). Use of preservatives, except sorbate salts, in canned pickled cucumbers is forbidden by ISIRI. Sorbate salts can be applied in production of canned pickled cucumbers maximally at 500 mg kg^{-1} for an 18 kg package. However, the results of this study show that sorbate was not detected in any samples of pickled cucumber. Maybe NaCl is used as another GRAS preservative in these products. According to regulations (ISIRI: No. 116 2008), NaCl can be applied to pickled cucumber in the range of 5–100 g.

Table 3. Recovery rates (%) for sodium benzoate and potassium sorbate in different Iranian products.

						Dairy products				
Analyte	Pickled cucumber	Canned tomato paste	Sour cherry jam	Carbonated soft drink	Fruit juice	UF-Feta cheese	Lighvan cheese	Lactic cheese	Yogurt	Doogh
Sodium benzoate	97.9	101.3	98.7	99.2	100.6	99.2	100.0	98.8	102.1	97.5
Potassium sorbate	96.0	100.7	99.0	99.1	99.7	101.8	101.3	99.2	98.9	98.4

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Table 4. Sodium benzoate and potassium sorbate (mg kg^{-1}) levels in the investigated Iranian samples.

		Sodium benzoate		Potassium sorbate	
Food matrix	Brand	Mean ± SD	Range	Mean ± SD	Range
Pickled cucumber	A	<1.9	<1.9	<1.5	<1.5
	B	<1.9	<1.9	<1.5	<1.5
	C	<1.9	<1.9	<1.5	<1.5
	D	<1.9	<1.9	<1.5	<1.5
Canned tomato paste	A	<1.9	<1.9	<1.5	<1.5
	B	57.6 ± 10.2	73.2–42.3	<1.5	<1.5
	C	42.7 ± 4.0	37.0–50.2	<1.5	<1.5
	D	26.9 ± 3.2	20.9–31.1	49.4 ± 3.8	42.2–55.3
Sour cherry jam	A	<1.9	<1.9	<1.5	<1.5
	B	<1.9	<1.9	<1.5	<1.5
	C	<1.9	<1.9	<1.5	<1.5
	D	<1.9	<1.9	<1.5	<1.5
Carbonated soft drink	A	114.8 ± 7.9	102.8–125.0	<1.5	<1.5
	B	<1.9	<1.9	68.5 ± 5.6	54.8–73.3
	C	102.3 ± 9.2	83.2–110.8	<1.5	<1.5
	D	92.6 ± 4.6	84.3–99.0	<1.5	<1.5
Fruit juice	A	<1.9	<1.9	<1.54	<1.5
	B	<1.9	<1.9	113.8 ± 22.3	78.7–141.6
	C	<1.9	<1.9	233.3 ± 50.2	123.4–284.3
	D	213.2 ± 38.6	127.9–288.5	<1.5	<1.5
Dairy products					
UF-Feta cheese	A	64.3 ± 14.3	39.8–80.0	<1.5	<1.5
	B	37.6 ± 12.2	27.6–68.9	43.3 ± 3.3	37.2–48.7
	C	54.4 ± 19.2	18.4–91.2	37.2 ± 26.7	<1.5–92.1
	D	46.2 ± 12.3	37.5–80.1	<1.5	<1.5
Lighvan cheese	A	31.5 ± 8.5	21.8–44.6	29.6 ± 26.1	<1.5–61.5
	B	27.6 ± 6.1	16.7–35.1	49.2 ± 19.2	<1.5–66.1
	C	43.9 ± 15.0	27.3–84.1	<1.5	<1.5
	D	35.2 ± 12.8	27.0–71.3	<1.5	<1.5
Lactic cheese	A	14.9 ± 2.8	11.9–20.4	<1.5	<1.5
	B	17.6 ± 2.4	12.4–21.0	<1.5	<1.5
	C	21.5 ± 2.6	16.7–25.6	<1.5	<1.5
	D	15.3 ± 2.7	12.0–21.8	<1.5	<1.5
Yogurt	A	19.6 ± 3.9	15.4–28.9	36.2 ± 33.8	<1.5–84.3
	B	32.0 ± 8.0	15.7–43.1	<1.5	<1.5
	C	28.3 ± 3.6	20.1–31.9	<1.5	<1.5
	D	37.3 ± 6.6	25.3–47.6	<1.5	<1.5
Doogh	A	22.2 ± 2.6	18.9–26.4	83.8 ± 10.9	62.1–95.9
	B	20.0 ± 1.8	18.2–24.4	32.0 ± 16.7	<1.5–63.4
	C	19.4 ± 2.4	14.9–22.4	<1.5	<1.5
	D	21.5 ± 4.2	17.7–30.0	<1.5	<1.5

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The detection of benzoate and sorbate in canned tomato paste showed that 75% of 40 samples were positive for benzoate with the range of 20.9–73.2 mg kg^{-1}. Moreover, according to Table 4, only one brand contained sorbate, which is 25% of the tomato paste samples. According to the ISIRI (No. 761 2012), any use of preservatives is prohibited in canned tomato paste. However, 75% of these samples were positive for at least one of these preservatives. These results show that some producing factories do not follow the standards. Therefore, some arrangements are needed for more supervision and control of food products. Cakir and Cagri-Mehmetoglu (2013) analysed tomato paste samples for benzoic acid and sorbic acid by using HPLC in Turkey. Their report showed that 5 out of 20 tomato paste samples contained benzoic acid with a mean of 202.5 mg kg^{-1} and 3 of these 20 samples contained a mean of 37.4 (<LOD–499.3) mg kg^{-1} sorbate.

Results of this study show that sour cherry jam samples did not contain benzoate and sorbate, which is not acceptable according to ISIRI (ISIRI: No. 214 1995). There are not any studies about the detection of benzoate and sorbate in sour cherry jam. However, there are some findings in other jams like quince. Ferreira et al. (2000) analysed quince jams in the Portuguese market for the occurrence of the benzoic and sorbic acid. They reported that all samples of quince jam contained benzoic acid with the

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concentration range from 413.9 ± 10.4 to 1501 ± 4.2 mg kg^{-1}. Only two brands contained sorbic acid. Koyuncu and Uylaşer (2009) also detected benzoic and sorbic acid in Turkish food such as jam using HPLC. They reported that the levels of benzoic and sorbic acid in the analysed jams were in the range of < LOD to 375 mg kg^{-1} (mean: 139.5 ± 41.7 mg kg^{-1}) and < LOD to 402 mg kg^{-1} (mean: 145.5 ± 44.7 mg kg^{-1}), respectively.

Average benzoate and sorbate concentrations in four brands of soft drinks were determined and given in Table 4. The results show that three brands contained benzoate at mean values of 103.2 ± 11.7 mg kg^{-1} with the range 83.2–125.0 mg kg^{-1}. Sorbate was detected in only one brand, at the mean of 68.5 ± 5.6 mg kg^{-1}. These results show that benzoate and sorbate are common preservatives in carbonated soft drinks to prevent the growth of yeasts, moulds and bacteria. These preservatives are allowed by ISIRI at maximum levels of 150 mg kg^{-1} for benzoate and sorbate each (ISIRI: No. 1250 2012). However, they can be harmful to human health when present at levels higher than permitted safety levels. Metabolic acidosis, convulsions, hyperpnoea, allergic reactions in experimental animals and in humans are some adverse effects, which are described in studies by Tfouni and Toledo (2002) and Wen et al. (2007). In this study, 90% and 45% of all samples were positive for benzoate and sorbate, respectively, which is not permitted by the legislation enforced in Iran. Thus, strict control by the food inspectors is needed in food production industries.

Whereas one brand did not contain any preservatives, at least one preservative was determined in three brands of fruit juice. Mean benzoate and sorbate concentrations in all fruit juice samples were 213.2 ± 38.6 and 173.6 ± 71.9 mg kg^{-1}, respectively. Sodium benzoate and potassium sorbate are preservatives that are commonly used in processed fruit juices to inhibit mold growth, prevent spoilage and preserve freshness. Under the provisions set by ISIRI in Iran, benzoate and sorbate cannot be used as a preservative in juices. Thus, all brands except one contained at least one preservative which is not allowed (ISIRI: NO. 14435 2012). There are some studies which reported the detection of benzoate and sorbate in various juices. Pylypiw and Grether (2000) analysed apple and grape juices and reported that these juices did not contain benzoate. However, they contained 0.013–0.035% and 0.023–0.045% sodium sorbate, respectively. In another study, Cakir and Cagri-Mehmetoglu (2013) reported that only 1 of 23 fruit juice samples contained 181.4 mg kg^{-1} benzoate, which is not permitted in Turkey. Tfouni and Toledo (2002) also detected benzoate and sorbate with mean levels of 495 and 51 mg kg^{-1} in fruit juice samples, respectively.

All dairy samples contained benzoate, with mean concentrations and ranges from 50.6 ± 17.4 (27.6–91.2), 34.6 ± 12.3 (16.7–84.1), 17.3 ± 3.7 (11.9–25.6), 29.3 ± 8.6 (15.4–47.6) and 20.8 ± 3.0 (14.9–30.0) mg kg^{-1} in UF-Feta cheese, Lighvan cheese, lactic cheese, yogurt and doogh, respectively (Table 4). In addition, sorbate was detected in two brands of UF-Feta cheese, Lighvan cheese and doogh and in one brand of yogurt. It was not detected in lactic cheese. However, preservatives were not noted on the labels of these products. El-Ziney (2009) analysed many types of cultured dairy products, such as yogurt and cheeses, for benzoic and sorbic acid by GC-MS and reported benzoic acid to occur as by-product of microbial degradation in dairy products, so it can appear naturally. Esfandiari et al. (2013) reported a less extensive study on the presence of benzoate and sorbate in doogh, with a mean concentration of 5.9 mg kg^{-1} for benzoate. There are differences from our method, whereas they apply methanol for extraction of benzoate from dairy products. However, benzoate is water soluble and it extracts better with a method which applies water. In another study, many types of yogurts have been found to contain natural levels of benzoic acid (Urbiene & Leskauskaite 2006). In other studies, the minimum benzoate concentrations reported in doogh were 18.3 mg kg^{-1} (Akbari-adergani et al. 2013), whereas Mazdeh et al. (2014) reported a maximum level of benzoate, produced naturally in doogh, of 30 mg kg^{-1}. Additionally, Javanmardi et al. (2015), in another Iranian study, reported 92.5% of the investigated samples were positive for benzoic acid with levels ranging between 3.5 and 1520 µg mL^{-1}, while for sorbic acid 50.3% of the samples were positive in a range of 1.8–2127 µg mL^{-1}.

All together, these reports obviously show that there is naturally some benzoate present in dairy products, as there were not any samples reported without benzoate. This phenomenon is long since known (Sieber et al. 1995) and attributed to hippuric acid, which is naturally present in milk. Recently Rukkwamsuk et al. (2012) reported lactic acid bacteria even to convert hippuric acid into benzoic acid during milk collection and storage, so the latter compound could be considered as a natural component of milk and milk products. Iammarino et al. (2011) tested 100 cheese samples (hard, semi hard, soft, semi soft, acid curd and pre-packed cheeses) for the maximum concentration of benzoate that is naturally present and therefore permitted in cheese. They estimated for benzoic acid a maximum admissible limit of 40 mg kg^{-1} in cheese. Other studies report the use of both preservatives in foods in other countries. For example, Ucla P et al. (2013) showed that only 21% of processed food products where use of these preservatives is authorised contained these two preservatives in Turkey.

Benzoic acid and sodium benzoate are generally GRAS preservatives in the United States and are allowed at levels up to a maximum of 0.1% (Code of Federal Regulations, 1977/1988; Title 21, Secs. 184.1021 and 184.1733). In the European Union the maximum level of

benzoate and sorbate in dairy-based drinks is 150 and 300 mg kg^{-1}, respectively (European Commission 2008). In addition, the usage of additives in food products must be labelled on packages according to this regulation. In most other countries, the maximum allowed quantities generally range between 0.15% and 0.25%, but in some other countries, benzoic acid is not considered as a food additive (Davidson et al. 2005). While sodium benzoate could be used for preservation of products such as soft drinks, its use is forbidden in dairy products by Iranian national standards (ISIRI: No.2453 2008; ISIRI: No. 695 2008; ISIRI: No. 2344 2008). The results of this study show that benzoate can be formed in cultured dairy products such as doogh, yogurt and cheese, naturally. For this reason it would be advisable to define a natural level, above which addition could be determined.

Conclusion

The proposed method, an isocratic reverse-phase HPLC method, is simple, rapid, selective and useful in the routine detection and determination of sodium benzoate and potassium sorbate in various types of food products. According to the result of this study, benzoate and sorbate are two commonly applied preservatives in Iran. As regards, it shows that 75% of the investigated samples were positive for at least one of these preservative at levels ranging from 11.9 to 288.5 mg kg^{-1}. Moreover, benzoate was detected in all dairy product samples and seems to be a common metabolite of lactic acid bacteria utilised during cheese, yogurt and doogh maturation. In conclusion, the variable levels of sorbate and benzoate applied in the control of microbial contamination of food are indicative of manufacturing practices of food processors. Closer monitoring of additives levels by regulatory agencies, application of good manufacturing practices (GMP) and better control of environmental conditions during storage and retail are warranted to reduce/eliminate food additives usage in food production as to assure human safety.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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