Development and Validation of a New Isocratic RP-HPLC Method for Simultaneous Determination of Sodium Metabisulfite and Sodium Benzoate in Pharmaceutical Formulation

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A simple and convenient reversed-phase high-performance liquid chromatography (RP-HPLC) method for simultaneous separation, identification, and determination of sodium metabisulfite and sodium benzoate in pharmaceutical formulation has been developed and validated. Chromatographic separation was achieved on RP column Zorbax Extend C-18 (150 × 4.6 mm i.d., 3.5 μm particles), and mixture of 0.1% phosphoric acid and acetonitrile in the ratio 62:38 (v/v) was used as a mobile phase. The flow rate was set at 1.0 mL/min with detection wavelength of 275 nm. The method was successfully validated according to International Conference on Harmonization (ICH) guidelines acceptance criteria. The method is selective, as no interferences were observed at retention times corresponding to these analytes. Results of regression analyses (r) and statistical insignificance of calibration curve intercepts (p) proved linearity of the method in defined concentration ranges for sodium metabisulfite and sodium benzoate (0.05–0.15 mg/mL). Relative standard deviations calculated for both analytes in precision testing were below the limits defined for active pharmaceutical ingredients (analysis repeatability: <2%; intermediate precision: <3%). Recovery values were between 98.16% and 101.94%. According to results of robustness testing, chromatographic parameters are not significantly influenced by small variation of acetonitrile content in mobile phase, column temperature, and flow rate. Finally, the method was applied for quantitative determination of investigated preservatives in real sample analysis.

Keywords: RP-HPLC, sodium metabisulfite, sodium benzoate, pharmaceutical formulations

Introduction

The analysis of preservatives in commercial pharmaceutical products is particularly important for both quality assurance and consumer protection. International Conference on Harmonization (ICH) guidance recommends that the finished product shelf-life specification should also include preservative content and limits for antimicrobial preservative present [1].

The antioxidants are substances with lower oxidation potential compared to that of active substances they protect. Sulfureous compounds are largely used as antioxidants in food products. Some sulfitic species, especially sodium metabisulfite, have been used in pharmaceutical formulations. Sodium metabisulfite is used as an antioxidant in oral, parenteral, and topical pharmaceutical formulations, at concentrations of 0.01–1.0% w/v. Primarily, sodium metabisulfite is used in acidic preparations, where it undergoes an instantaneous hydrolysis to sodium bisulfite and further to a weak sulfurous acid. Sodium metabisulfite also has some antimicrobial activity, which is greatest at acid pH, and may be used as a preservative in oral preparations such as syrups [2]. A necessity for a strict control of sodium metabisulfite usage results from its adverse effect on health cytotoxicity and mutagenicity of this compound is confirmed [3–6].

Up to date, different analytical methods have been reported for determination of sodium metabisulfite based on high-performance liquid chromatography (HPLC) [7], ion chromatography with suppressed conductivity detection [8, 9], flow injection coulometry [10], amperometric method [11], capillary zone electrophoresis with indirect detection [12], and isotachophoretic method [13].

Sodium benzoate is generally used as a chemical preservative to prevent alteration or degradation caused by microorganisms.

In acidic media (pH 2–5), sodium benzoate exhibits inhibitory activity against a wide range of fungi, yeasts, molds, and bacteria and is widely used in variety of products, such as cosmetics and pharmaceuticals, but more commonly in foods. It is used in concentrations of 0.02–0.5% in oral medicines, 0.5% in parenteral products, and 0.1–0.5% in cosmetics [2].

Its toxicity is generally low owing to fast hydrolysis in vivo to parent acid, which is rapidly conjugated and excreted [2]. However, excessive intake of preservatives might be potentially harmful to the consumers [14].

Most of the literature data concerns determination of sodium benzoate in a mixture with drugs or other preservatives. Preservatives are most commonly determined in samples of food and beverage [15–18], pharmaceuticals [19–22], and cosmetics [23, 24]. The most frequently used technique in preservative analysis is HPLC. The literature also contains other analytical techniques for determination of sodium benzoate such as ultraviolet (UV) spectroscopy [25], near-infrared reflectance spectroscopy [26], chiral ligand exchange capillary electrophoresis [27], fluorescence polarization immunoassay [28], and electrochemistry using ion-selective electrodes [29].

To our knowledge, there is no report about simultaneous determination of sodium metabisulfite and sodium benzoate in pharmaceutical products. The aim of this work was to develop fast, simple, selective, and easy-to-use method for simultaneous control of those two preservatives in pharmaceuticals.

Experimental Part

Chemicals and Reagents. HPLC gradient grade acetonitrile was purchased from JT Baker (Phillipsburg, NJ, USA) and orthophosphoric acid from Merck (Darmstadt, Germany).

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Working standards of sodium metabisulfite (99.56%) and sodium benzoate (99.43%) were produced by Centrohem (Stara Pazova, Serbia). Klozan® placebo contains ethylenediaminetetraacetic acid (EDTA) disodium salt, trisodium citrate dihydrate, citric acid monohydrate, propylene glycol, and glycerol anhydrous. Klozan® suspension for injection was produced by Evrolek-Pharmacija d.o.o. (Šabac, Serbia).

Chromatographic Conditions. HPLC analysis was performed on chromatograph Dionex UltiMate 3000 system (Thermo Fisher Scientific, Germering, Germany) equipped with quaternary pump, autosampler, and DAD detector. Intermediate precision was tested on chromatograph Agilent 1200 (Agilent Technologies, Palo Alto, CA, USA) equipped with binary pump, manual injector (20 μL loop), and DAD detector. The column used was Zorbax Extend (150 × 4.6 mm i.d., 3.5 μm particles), thermostated at 25 °C. Mobile phase consisted of 0.1% phosphoric acid and acetonitrile (62:38 *v/v*). The flow rate was 1 mL/min, and detection was performed at 275 nm.

Preparation of Solution

Solvent. One milliliter of ortophosphoric acid 85% was transferred to 1000 mL volumetric flask and diluted with deionized water.

Placebo Stock Solution Spiked with Active Pharmaceutical Ingredients. An accurately weighed amount of citric acid (70 mg), sodium citrate dihydrate (47 mg), EDTA disodium salt (10 mg), levamisole hydrochloride (500 mg), glycerol anhydrous (430 mg), and propylene glycol (6.15 mg) was transferred to 10 mL volumetric flask containing 2.3 mL of water for injection. Every additional substance was transferred to a volumetric flask only after dissolving the previous one. Finally, accurately weighed amount of closantel (500 mg) was added and the mixture was sonicated for 15–20 min. Prepared and sonicated solution was centrifuged and filtered through polytetrafluoroethylene (PTFE) membrane filter (0.45 μm). One milliliter of supernatant solution was transferred to a 10 mL volumetric flask and diluted with the solvent.

Placebo Working Solution. One milliliter of placebo stock solution was transferred to 10 mL volumetric flask and diluted with the solvent.

Standard Stock Solution. An accurately weighed amount of sodium metabisulfite (10 mg) and sodium benzoate (10 mg) were transferred to 10 mL volumetric flask, and 7 mL of solvent was added, sonicated for 5–10 min, diluted with the solvent, and filtered through PTFE membrane filter (0.45 μm).

Standard Working Solution. One milliliter of standard stock solution was transferred to a 10 mL volumetric flask and diluted with the solvent.

Placebo Solution Spiked with Standard Solution of Sodium Metabisulfite and Sodium Benzoate. One milliliter of standard stock solution and 1 mL of placebo working solution were transferred to a 10 mL volumetric flask and diluted with the solvent.

Method Validation. The performance and validation of the method were evaluated considering the guidelines of the ICH [30] by determining selectivity, linearity, precision, accuracy, and robustness.

Selectivity. Selectivity testing of this method was performed by injecting three solutions under optimal chromatographic conditions: Klozan® placebo solution, Klozan® placebo solution spiked with standard solution of sodium metabisulfite and sodium benzoate, and working standard solution of tested substances. Selectivity was tested by examining the chromatogram of Klozan® placebo at retention times corresponding to sodium metabisulfite and sodium benzoate.

Linearity. For the construction of calibration curves, five solutions of mixture of analytes (sodium metabisulfite and sodium benzoate) were prepared by diluting stock standard solution with the solvent. Linearity for both compounds was tested in the range

of 0.05–0.15 mg/mL. Each point of the calibration graph corresponded to the mean value obtained from three independent measurements.

Precision. Precision was assessed in terms of repeatability and intermediate precision. Six solutions of the mixture of analytes at 100% of the test concentration (0.1 mg/mL) were prepared for analysis repeatability and injected, and relative standard deviation was calculated. For intermediate precision, six solutions were prepared as described for analysis repeatability and this test was performed by another analyst, another day, on another HPLC chromatograph and with different set of reagents.

Accuracy. Accuracy was tested at three concentration levels (80%, 100%, and 120%) by adding three different volumes of stock standard solution (800, 1000, and 1200 μL) into three 10 mL volumetric flasks, each containing previously measured 1 mL of placebo stock solution and 4 mL of the solvent. The solutions were diluted, filtered, and injected. Concentrations of sodium metabisulfite, as well as sodium benzoate were 0.08 mg/mL (80%), 0.1 mg/mL (100%) and 0.12 mg/mL (120%).

Robustness. Robustness was tested by small variations of selected parameters: acetonitrile content in mobile phase, column temperature, and flow rate. Working standard solution was prepared by diluting stock standard solution with the solvent to obtain concentration of 0.1 mg/mL for both analytes. Placebo solution spiked with standard solution of sodium metabisulfite and sodium benzoate was prepared by adding 1 mL of stock standard solution and 1 mL of placebo stock solution into 10 mL volumetric flask and diluting with the solvent. Working standard solution and placebo solution spiked with standard solution of sodium metabisulfite and sodium benzoate were injected under each of tested chromatographic conditions, and peak areas of tested analytes were monitored.

Real Sample Testing. Five milliliters of Klozan® suspension for injection was centifuged (5 min on 4000 rpm), and supernatant liquid was filtered through PTFE membrane filter (0.45 μm). One milliliter of obtained filtrate was transferred to 10 mL volumetric flask, diluted with the solvent, and injected.

Results and Discussion

Method Development and Optimization. Optimum conditions, which are necessary for the quantitative analysis of compounds with maximum sensitivity, were established by selection of stationary phase, varying mobile phase composition and pH of the mixed solvent system, and observing their effects on capacity factor (k), peak width, and symmetry. Acetonitrile is selected as organic solvent because of higher elution strength. In order to study the effect of mobile phase pH on chromatographic separation, the effect of pH on the retention time of each analyte was investigated over the range of 2.0–6.0. The results obtained showed that the resolution of sodium metabisulfite decreases as the pH increases which can be explained with the fact that sodium metabisulfite exsists in anionic form in acidic medium [31]. Therefore, a pH 3.0 was selected for further investigation. The 1.0 mL/min flow rate was selected to achieve the separation of peaks. It was found that ambient temperature is an appropriate temperature with respect to peak separation and shape. Based on the UV spectra of sodium metabisulfite and sodium benzoate, 275 nm was found to be appropriate wavelength for the determination of those two preservatives. From the mobile phase selection study, the optimized HPLC parameters were as follows: flow rate, 1.0 mL/min; column temperature, 25 °C; and isocratic program with a mixture of 0.1% phosphoric acid and acetonitrile in the ratio of 62:38 (*v/v*) as a mobile phase. The chromatographic separation was performed on various columns including RP8, RP18, cyano, and amino HPLC columns. Retention time was

not affected in the case of sodium metabisulfite, while k for sodium benzoate increased with increasing polarity of stationary phase. Therefore, RP18 column was selected in order to achieve high resolution between peaks of analyzed analytes.

Method Validation

Selectivity. The method is selective for simultaneous determination of sodium metabisulfite and sodium benzoate, as no interferences were observed at retention times corresponding to these analytes (Figure 1).

Linearity. Results of regression analyses ($r = 0.9995$ for sodium metabisulfite and $r = 0.9999$ for sodium benzoate) as well as statistical insignificance of calibration curve intercepts ($p > 0.05$) proved linearity of the method in defined concentration ranges (Table 1).

Table 1. Linearity parameters of the proposed method

	Sodium metabisulfite	Sodium benzoate
Range (mg/mL)	0.051–0.165	0.052–0.157
a	54.7587	110.9967
b	−0.2883	−0.2127
r	0.9995	0.9999
p	0.09703	0.283904
SE intercept	0.120795	0.14539
SE slope	1.041905	1.312963

a, slope; b, intercept; r, correlation coefficient; p, statistical significance of calibration curve intercept ($p > 0.05$); SE intercept, standard error of intercept; and SE slope, standard error of slope.

Precision. Precision was tested at concentration level of 100%. Relative standard deviations calculated for sodium
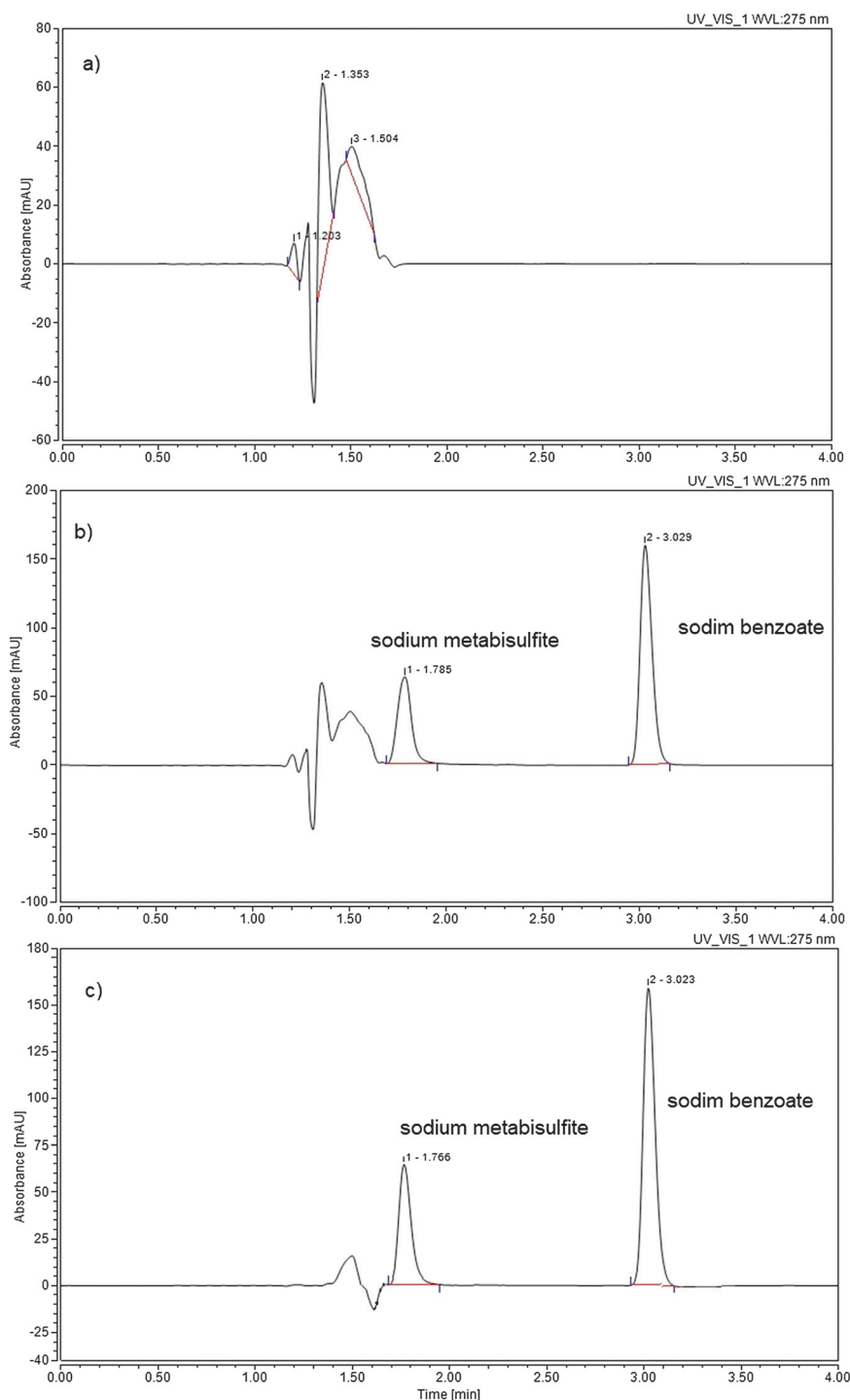
Figure 1. Chromatograms: a) Klozan® placebo solution; b) Klozan® placebo solution spiked with standard solution of sodium metabisulfite ($t_r = 1.766$) and sodium benzoate ($t_r = 3.024$) and c) standard solution of sodium metabisulfite and sodium benzoate

metabisulfite and sodium benzoate were below the limits defined for active pharmaceutical ingredients (analysis repeatability: <2%; intermediate precision: <3%) [27]. The results of precision testing are presented in Table 2.

Accuracy. Recovery for sodium metabisulfite and sodium benzoate was tested at three levels (80%, 100%, and 120%) in

regard to the concentration in solution prepared for the determination of analytes. These values were between 98.16% and 101.94%, which is within the required range for active pharmaceutical ingredients (98–102%) [32]. These results (Table 2) proved the method's accuracy for the simultaneous determination of sodium metabisulfite and sodium benzoate.

Robustness. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters provide an indication of its reliability during normal usage. Robustness was tested by small variations of selected parameters: acetonitrile content in mobile phase, column temperature, and flow rate. Standard working solution and placebo solution spiked with standard solution of sodium metabisulfite and sodium benzoate were injected according to chromatographic conditions presented in Table 3. The concentration of analyzed analyte was calculated according to Eq. (1).
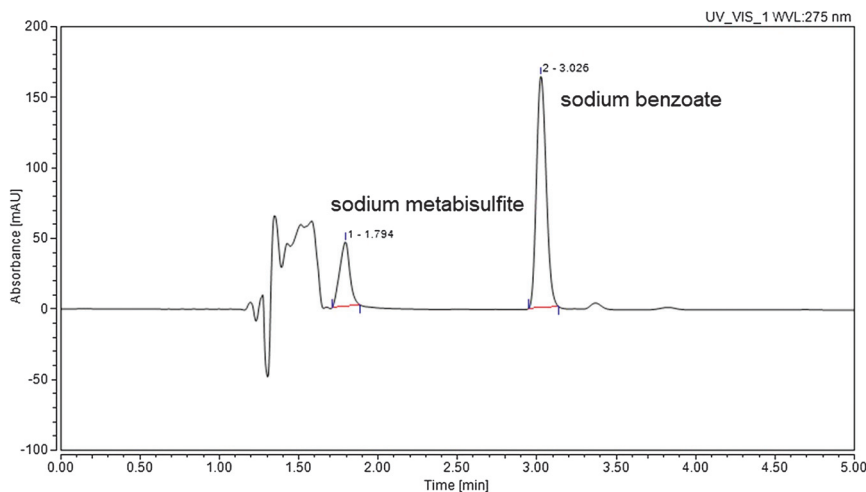
$$C_0 = (A_i/A_S) \times C_S \qquad (1)$$

A_i — peak area of analyzed compound in placebo solution spiked with standard solution of sodium metabisulfite and sodium benzoate

A_S — peak area of analyzed compound in standard working solution

Table 2. Results of precision and accuracy testing

	Sodium metabisulfite	Sodium benzoate
Precision		
Analysis repeatability		
Concentration of analyte (mg/mL)	0.0980	0.1015
Mean value (mg/mL)	0.0954	0.1024
Standard deviation (mg/mL)	0.001	0.0004
RSD (%)	1.03	0.43
Intermediate precision		
Concentration of analyte (mg/mL)	0.0940	0.1015
Mean value (mg/mL)	0.0926	0.1016
Standard deviation (mg/mL)	0.001	0.002
RSD (%)	1.37	1.64
Accuracy		
Concentration 1 (mg/mL)	98.16	101.52
Recovery (%)		
Concentration 2 (mg/mL)	98.84	99.90
Recovery (%)		
Concentration 3 (mg/mL)	99.06	101.94
Recovery (%)		

Concentration 1, 80%; concentration 2, 100%; and concentration 3, 120%.

Table 3. Results of robustness testing

Optimal chromatographic conditions					

Mobile phase: 0.1% phosphoric acid–CH₃CN, 62:38 (v/v)
Flow rate: 1.0 mL/min
Column temperature: 25 °C

		Sodium metabisulfite		Sodium benzoate	
		C_s (mg/mL)	C_o (mg/mL)	C_s (mg/mL)	C_o (mg/mL)
		0.098	0.0954	0.1015	0.1024
Variation		Sodium metabisulfite		Sodium benzoate	
		C_s (mg/mL)	C_o (mg/mL)	C_s (mg/mL)	C_o (mg/mL)
Acetonitrile content	34	0.098	0.0958	0.1015	0.1017
	Deviation (%)		0.41		0.69
	42	0.098	0.0969	0.1015	0.1020
	Deviation (%)		1.94		0.40
Variation		Sodium metabisulfite		Sodium benzoate	
		C_s (mg/mL)	C_o (mg/mL)	C_s (mg/mL)	C_o (mg/mL)
Flow rate	0.9	0.098	0.0959	0.1015	0.1027
	Deviation (%)		0.92		0.30
	1.1	0.098	0.0951	0.1015	0.1020
	Deviation (%)		0.10		0.40
Variation		Sodium metabisulfite		Sodium benzoate	
		C_s (mg/mL)	C_o (mg/mL)	C_s (mg/mL)	C_o (mg/mL)
Column temperature	22.5	0.098	0.0961	0.1015	0.1026
	Deviation (%)		1.12		0.19
	27.5	0.098	0.0952	0.1015	0.1028
	Deviation (%)		0.20		0.39

Figure 2. Chromatograms of Klozan® solution

C_S — concentration of analyzed compound in standard working solution (mg/mL)

C_0 — concentration of analyzed compound in placebo solution spiked with standard solution of sodium metabisulfite and sodium benzoate

The results of robustness testing are presented in Table 3. According to these results, it can be concluded that tested chromatographic parameters are not significantly influenced by selected factors because the obtained deviations were below the limit defined for precision of the method (±3%). Thus, robustness of developed method was confirmed.

Analysis of Pharmaceutical Dosage Form. Applicability in routine of this method was tested by analysis of Klozan® suspension for injection. The content of sodium metabisulfite was 95% (0.095 mg/mL) while the content of sodium benzoate was 103% (0.103 mg/mL). These results are in accordance with Klozan® suspension for injection specification. Chromatograms of Klozan® solution are presented in Figure 2.

Conclusion

The newly developed reversed-phase RP-HPLC method was found to be convenient for the simultaneous determination of sodium metabisulfite and sodium benzoate. The method provides selective quantification of those two preservatives within 3 min without interference from ingredient and placebo. Obtained validation parameters (selectivity, linearity, precision, accuracy and robustness) proved that the suggested method is convenient enough for routine determination of sodium metabisulfite and sodium benzoate in quality control laboratories.

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