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Journal of Pharmaceutical and Biomedical Analysis
31 (2003) 1059–1064
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Simultaneous analysis of metabisulfite and sulfate by CE with indirect UV detection. Application to and validation for a pharmaceutical formulation

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Received 16 October 2002; received in revised form 28 November 2002; accepted 28 November 2002

Abstract

Metabisulfite is used as an antioxidant agent in a number of pharmaceutical formulations. In order to quantify simultaneously both metabisulfite and its oxidation product (sulfate), a capillary zone electrophoretic (CZE) method with indirect UV detection was developed. Best results were achieved with a background electrolyte (BGE) constituted of 15 mM pyromellitic acid, 15 mM tris-(hydroxymethyl)-aminomethane and 0.2 mM tetradecyltrimethylammonium bromide at pH 8.3 and an applied electrical field of 123 V/cm in a 32.5 cm fused silica capillary. Indirect UV detection was performed at a wavelength of 225 nm. In order to validate this method, an internal standard (IS), namely ammonium formate, was used. Moreover, due to the high chloride concentration in the pharmaceutical formulation, conductivity was adjusted by adding sodium chloride into standard solutions to prevent matrix effect. Linearity and accuracy were successfully tested in a concentration range of 33.3–250 μg/ml for sodium metabisulfite and of 50–375 μg/ml for sodium sulfate. Method precision was determined on six samples each day. Thereby, relative standard deviations (R.S.D.) of 6% and 12–13% were obtained for intra-day and inter-day precision, respectively. Considering the instability of metabisulfite and its use as an antioxidant agent and not as an active principle, the method was accepted and used for routine analyses.

Keywords: Capillary electrophoresis; Indirect UV; Inorganic anions; Metabisulfite; Sulfate; Validation

1. Introduction

Sulfureous compounds are largely used as preservatives in food, under different forms (e.g. sulfur dioxide, sulfite, hydrogen sulfite, etc). In fact, these agents are used in food products for their antimicrobial and antioxidant activity [1,2]. More recently, some sulfitic species, especially

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0731-7085/02/$ - see front matter
doi:10.1016/S0731-7085(02)00651-9

sodium metabisulfite ($Na_2S_2O_5$) and potassium metabisulfite ($K_2S_2O_5$), have been used in pharmaceutical formulations for their antioxidant properties. Indeed, metabisulfite can be easily oxidized into sulfate, which prevents other compounds from oxidation. Thus, it is important to have at disposal an analytical method that allows to quantify both inorganic anions, metabisulfite and sulfate, in a pharmaceutical formulation.

In recent years, capillary zone electrophoresis (CZE) with indirect UV detection has been successfully applied to the analysis of many inorganic anions. Some recent reviews summarize the large number of applications of CZE for ion analysis [3–6]. This technique is an interesting alternative analytical technique to ion chromatography (IC) and presents some advantages in terms of efficiency, selectivity, speed of analysis and small solvent consumption [7–9].

To our knowledge, only a few articles have been dedicated to the analysis of sulfitic species (such as sulfite or metabisulfite) by CZE [10,11]. In this paper, a method is described for the simultaneous analysis of metabisulfite and sulfate in a pharmaceutical formulation. This method was validated according to SFSTP requirements [12] and successfully applied to the analysis of a pharmaceutical formulation. Moreover, the selectivity of this method for other inorganic and organic anions is briefly demonstrated.

2. Experimental

2.1. Chemicals

The tested pharmaceutical formulations are injectable solutions containing a local anesthetic and were kindly obtained from Sintetica S.A. (Mendrisio, Switzerland). Their main constituents were lidocaine HCl and sodium chloride, but they also contained epinephrine and sodium metabisulfite (to prevent epinephrine's oxidation). All other reagents and solvents were of analytical grade and purchased from Fluka (Buchs, Switzerland). Ultra-pure water was supplied by a Milli-Q RG unit from Millipore (Bedford, MA, USA).

The aqueous background electrolyte (BGE) was constituted of pyromellitic acid at 15 mM, tris-(hydroxymethyl)-aminomethane (Tris) at 15 mM and tetradecyltrimethylammonium bromide (TTAB) at 0.2 mM. The pH was adjusted to 8.3 with 0.1 M sodium hydroxide.

2.2. Instrumentation

CE experiments were performed using a $HP^{3D}CE$ system (Agilent Technologies, Waldbronn, Germany) equipped with a diode-array detector, an autosampler and a power supply able to deliver up to 30 kV. A CE CHEMSTATION software (Agilent Technologies) was used for CE control, data acquisition and data handling. The separation was performed in a fused silica capillary (Polymicro, Phoenix, AZ, USA) with an inner diameter of 50 μm and 32.5 cm total length (24 cm to the UV detector). All experiments were performed using a reversed polarity mode (cathode at the capillary inlet and anode at the capillary outlet). A constant voltage of 4 kV (ca. 123 V/cm) was applied throughout the analysis. The capillary was thermostated at 25 °C. Samples were kept at ambient temperature in the autosampler and injected by applying a pressure of 50 mbar for 2 s (ca. 5.3 ml injected).

Indirect UV detection was carried out at 225 nm with a bandwidth (bw) of 10 nm and a reference set at 350 nm (bw of 100 nm). When performing indirect UV detection, negative peaks were recorded. However, positive peaks allowed much better automatic integration results with the CE Chemstation. In order to monitor positive peaks by indirect-UV, detection and reference were switched (e.g. UV measurement was carried out at 350 nm with reference at 225 nm). For sake of clarity, indirect-UV detection is reported at 225 nm throughout this paper.

Before its first use, the fused silica capillary was sequentially rinsed with 1 M sodium hydroxide, 0.1 M sodium hydroxide, water and BGE for 3 min for each solvent. Between analyses, the capillary was flushed with BGE for 2 min. When not in use, the capillary was rinsed with 0.1 M sodium hydroxide, water and then dry stored. As the electrophoresis process alters the running

buffer pH by electrolysis and subsequently changes the electroosmotic flow (EOF), the separation buffer was refreshed every four runs.

2.3. Validation requirements

Validation was performed according to SFSTP requirements [12] on 3 days with independent preparation of BGE and samples each day. Standard (std) and reconstituted dosage formulation (rdf) solutions at five different concentration levels were prepared each day to demonstrate linearity and accuracy by statistic tests (i.e. Fischer and t tests). Moreover, six different rdf solutions were prepared each day to calculate intra-day relative standard deviation (R.S.D.) and inter-day R.S.D., which represent, respectively, repeatability and intermediate precision.

3. Results and discussion

3.1. Method development

In order to perform indirect UV detection, a buffer electrolyte with a strong UV absorption ion has to be selected. Apart from being a UV-absorbing agent, this compound and the analytes should have similar electrophoretic mobilities. Indeed, when electrophoretic mobilities are mismatched, significant peak tailing or fronting might be observed, since the electrodispersion phenomenon can be critical. Usually, methods for the analysis of sulfureous species (especially sulfate) use chromate as an absorbing agent [4,13–18], therefore, this strong oxidant was tested first. However, oxidation of metabisulfite into sulfate was observed, especially when the same sample vial was used for different runs. This phenomenon was probably due to a small contamination by the BGE during the injection process. By selecting another absorbing agent, this problem was resolved. Among the other tested agents, pyromellitate and salicylate were by far the ones, which offered the best results for metabisulfite and sulfate. In fact, pyromellitate and salicylate possess approximately the same electrophoretic mobility as metabisulfite and sulfate [5,10,11,19–21]. Both agents were further tested and, finally, pyromellitate was selected as the chromophoric anion in BGE, since slightly better quantitative results were obtained. Different pyromellitate concentrations were also tested and a concentration of 15 mM was selected. Lower concentrations produced sensitivity problems, while higher concentrations caused more baseline drift.

To obtain negatively charged metabisulfite and sulfate, BGE should be set at a high pH, since pK_{a2} of sulfite is 7.3. For that reason, Tris ($pK_a = 8.3$) was added to BGE for its buffering capacity as well as for its low conductivity. By working at pH 8.3 and with the anode at the capillary outlet, a high EOF was generated from the outlet towards the inlet, which considerably increased analysis time. In order to accelerate analysis by reversing the EOF direction, TTAB, which is a cationic surfactant, was added below its critical micelle concentration (CMC) to BGE [16].

The applied voltage was set at a low value (i.e. 4 kV), which induced a current of about 12 μA. Higher voltage induced a tremendous baseline drift in the separation window of interest. The major drawback of such a low voltage is a longer analysis time (i.e. 8.5 min), but a higher voltage provided worse quantitative results.

3.2. Selectivity and sensitivity

To test the selectivity, all pharmaceutical formulation constituents were injected separately. These constituents were lidocaine chlorhydrate, sodium chloride, epinephrine and sodium metabisulfite. Apart from chloride and metabisulfite, all other constituents were either neutral or positively charged at a working pH of 8.3, and were, therefore, not detected after 10 min. Moreover, sodium sulfate (i.e. metabisulfite degradation product) and an internal standard (IS), namely ammonium formate, were also injected and no interference occurred. Fig. 1 shows the analysis of a pharmaceutical formulation after partial oxidation of metabisulfite into sulfate. It is noteworthy that chloride, which is the most abundant compound detected in this example presented strong peak fronting. While it did not interfere directly with analytes of interest, it could induce some

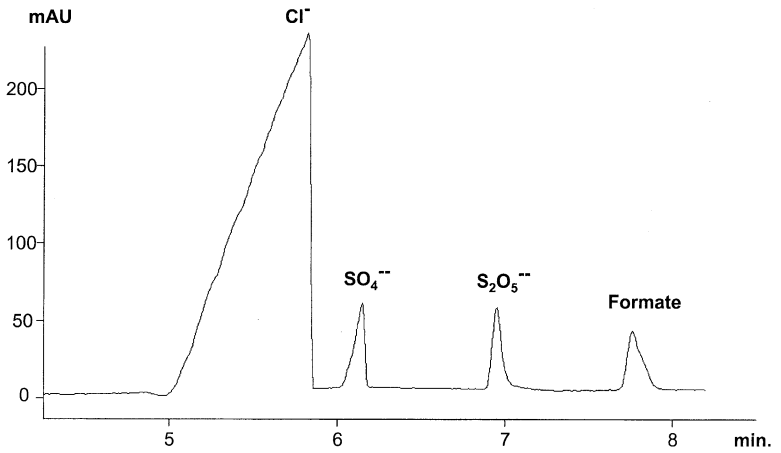

Fig. 1. Separation of sodium chloride (2500 μg/ml), sodium metabisulfite (150 μg/ml), sodium sulfate (150 μg/ml) and formate ammonium (IS, 150 μg/ml) in a pharmaceutical formulation. Capillary of 32.5 cm (fused silica, e.l. 24 cm, 50 μm i.d.), BGE containing 15 mM tris, 15 mM pyromellitic acid, 0.2 mM TTAB, adjusted to pH 8.3 with NaOH, negative voltage of 4 kV, 25 °C, injection by pressure 100 mbar s, indirect UV detection at 225 nm.

problems for further quantitative results, as discussed in Section 3.3. Moreover, method selectivity was also tested for other inorganic and organic anions. Tested anions were bromide, chloride, thiosulfate, sulfate, chlorate, metabisulfite, citrate, tartrate, phosphate and carbonate (Fig. 2).

It is to be noted that electrodispersion is a phenomenon of utmost importance. Actually, both sulfate and sodium chloride exhibited peak fronting, while both metabisulfite and formate showed peak tailing (Fig. 1). This confirmed that pyromellitate possesses an electrophoretic mobility ranging between sulfate and metabisulfite [19]. For small inorganic anions, the electrodispersion phenomenon is more critical than with CE applications dedicated to larger organic compounds such as pharmaceutical compounds or macromolecules. However, this phenomenon can be significantly diminished by injecting a smaller quantity of analyte, as shown in Fig. 2. Nevertheless, operating at a low concentration could be detrimental to method precision, due to the small peak areas obtained. A compromise had to be found in terms of efficiency and sensitivity, and concentration between 30 and 500 μg/ml were selected to perform linearity study for both anions.

For both sodium metabisulfite and sodium sulfate, limit of detection (LOD) and limit of quantification (LOQ) were estimated at 3 and 10 μg/ml, respectively (signal to noise ratio of 3 and 10). This sensitivity was largely sufficient to carry

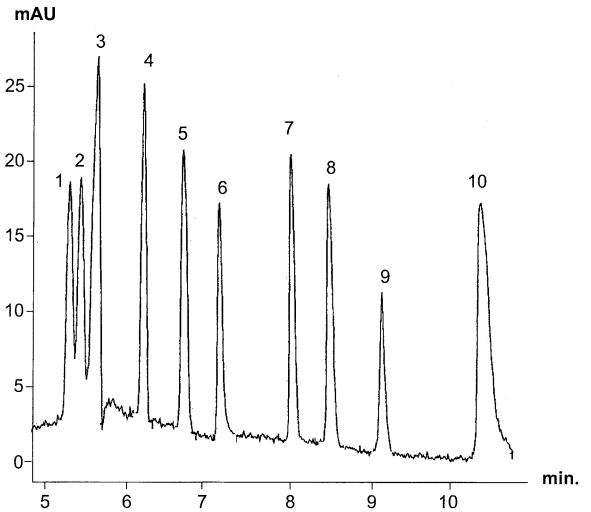

Fig. 2. Method selectivity tested with different anions (experimental conditions as stated in Fig. 1), all anions were prepared from their sodium salt at different concentration to obtain approximately equivalent peak height: (1) Br^- (100 μg/ml NaBr); (2) Cl^- (50 μg/ml); (3) $S_2O_3^{2-}$ (200 μg/ml); (4) SO_4^{2-} (75 μg/ml); (5) ClO_3^- (100 μg/ml); (6) Metabisulfite (50 μg/ml); (7) Citrate (75 μg/ml); (8) Tartrate (75 μg/ml); (9) Phosphate (25 μg/ml); (10) Carbonate (75 μg/ml).

Table 1
Recovery and precision data for the simultaneous analysis of sodium metabisulfite and sodium sulfate, using ammonium formate as IS

	Metabisulfite	Sulfate
Determination coefficient (r^2) for std and rdf samples	0.969/0.990	0.976/0.992
Recovery and confidence interval $\bar{x} \pm t^*_{(0.05;N-1)} S_x/\sqrt{N}$	100.2% ± 3.2	100.6% ± 3.1
Intra-day precision	R.S.D. = 6.0 (%)	R.S.D. = 5.6 (%)
Day-to-day precision	R.S.D. = 12.9 (%)	R.S.D. = 12.1 (%)

$\bar{x}$ and S_x corresponds to the recovery mean and its standard deviation; N is the number of variables ($N = 15$); $t_{(0.05;N-1)}$ is the appropriate t table value at 95% confidence level ($t_{(0.05;14)} = 2.145$).

out the analyses of the selected pharmaceutical formulations.

3.3. Quantitative results

Besides selectivity and sensitivity, other parameters have to be evaluated in the validation process. According to SFSTP guidelines [12], linearity, accuracy, intra-day and day-to-day precision were tested on three different days.

3.3.1. Linearity and accuracy

Linearity and accuracy were tested on 3 days at five concentration levels between 33.3 and 250 μg/ml for sodium metabisulfite and between 50 and 375 μg/ml for sodium sulfate on std and rdf solutions. These concentrations corresponded to approximately 15–100% of metabisulfite initially present in the formulation solution and to approximately 15–100% of metabisulfite oxidized into sulfate.

As mentioned above, validation was performed on 3 days according to SFSTP guidelines [12]. Meanwhile, after day 1, results were carefully examined to detect any analytical problem in the method. This is called the prevalidation step. By comparing std and rdf slopes (t-test), a significant matrix effect was noticed. This effect was attributed to the high concentration of chloride in rdf samples, inducing different electrical fields in both sample zones. Consequently, the peak shapes of metabisulfite and sulfate were different in std and rdf samples and induce inaccurate results. Therefore, sodium chloride was added to std samples to obtain approximately the same conductivity in both std and rdf samples, and, thereby, prevent matrix effect. Thus, pharmaceutical formulations could be directly injected into the capillary after a simple dilution (1:1) in an aqueous solution containing the IS.

In order to confirm linearity and accuracy, different statistical tests were performed on std and rdf solutions [12]. Calculated determination coefficients (r^2) for different slopes are reported in Table 1 and their values are rather good, especially in regard with method precision, which will be discussed in next paragraph. Besides, Fisher tests ($F_1/F_2/F_3$) were performed to attest linearity and compare std and rdf variances, and t tests were carried out to confirm accuracy. All tests were positive for both compounds and, thereby, attested method linearity and accuracy. After demonstrating that variances (s^2) at each concentration level were not significantly different through a Cochran test (C-test), recoveries and confidence intervals were calculated using all 15 points (three points per five tested concentration levels) and reported in Table 1.

3.3.2. Intra-day and day-to-day precision

Precision was tested on six rdf samples at 150 μg/ml of sodium metabisulfite (corresponding to 60% of sodium metabisulfite initially present in formulation) and 225 μg/ml of sodium sulfate (corresponding to 60% of sodium metabisulfite oxidized into sodium sulfate). Determined R.S.D. values on intra-day precision for both sodium metabisulfite and sodium sulfate were about 6%, while R.S.D. values on inter-day precision were about 12–13% (Table 1).

Obtained R.S.D. values were superior than those commonly obtained by CE–UV (about 1–

2% R.S.D.), which was attributed to different reasons. First, indirect UV detection is generally less precise than direct UV detection. In addition, as described above, the high sample concentration of anions (especially chloride) was responsible for peak tailing and fronting. Moreover, whatever the analytical technique used, quantifying an unstable compound and its degradation product impairs method precision. The latter cause was probably the most influent on precision data. However, the analysis of an antioxidant and its degradation product does not require the same analytical precision as for a pharmaceutical drug. Thus, the validated method was accepted to quantify the concentrations of sodium metabisulfite and sodium sulfate in pharmaceutical formulations.

4. Conclusion

A CZE method with indirect UV detection was successfully developed for the separation and quantification of sodium metabisulfite and its degradation product, namely sodium sulfate, in a pharmaceutical formulation. During method development, the choice of the appropriate chromophoric agent in BGE was of utmost importance to take into account the electrodispersion phenomenon. Unfortunately, chromate, which is the most commonly used agent to analyse sulfitic species, was found inappropriate for metabisulfite's quantification, since it rapidly oxidized metabisulfite into sulfate. Therefore, another chromophoric agent was selected, namely pyromellitate. After choosing a suitable BGE, the method was validated according to SFSTP requirements, and fulfilled all exigencies. Recovery and confidence limits were calculated on the tested concentration ranges, generating good results for both sodium metabisulfite and sulfate. Besides, the obtained intra-day and inter-day R.S.D. values were about 6 and 12%, respectively. These precisions were fully suitable for the quantification of an antioxidant and its degradation product, and consequently, the validated method was used in routine analysis.

References

[1] J. Sádecká, J. Polonský, J. Chromatogr. A 834 (1999) 401–417.
[2] J. Sádecká, J. Polonský, J. Chromatogr. A 880 (2000) 243–279.
[3] C.A. Lucy, J. Chromatogr. A 850 (1999) 319–337.
[4] D. Kaniansky, M. Masár, J. Marák, R. Bodor, J. Chromatogr. A 834 (1999) 133–178.
[5] J. Fritz, J. Chromatogr. A 884 (2000) 261–275.
[6] P. Morin, Analusis 27 (1999) 107–120.
[7] P.R. Haddad, P. Doble, M. Macka, J. Chromatogr. A 856 (1999) 145–177.
[8] P.R. Haddad, J. Chromatogr. A 770 (1997) 281–290.
[9] C.A. Lucy, J. Chromatogr. A 739 (1996) 3–13.
[10] S. Motellier, M. Descostes, J. Chromatogr. A 907 (2001) 329–335.
[11] S. Motellier, K. Gurdale, H. Pitsch, J. Chromatogr. A 770 (1997) 311–319.
[12] J. Caporal-Gautier, J.M. Nivet, S.T.P. Pharma Pratiques 2 (4) (1992) 205–226.
[13] P. Jandik, W.R. Jones, J. Chromatogr. 546 (1991) 431–443.
[14] W.R. Jones, P. Jandik, J. Chromatogr. 546 (1991) 445–458.
[15] S.M. Cousins, P.R. Haddad, W. Buchberger, J. Chromatogr. A 671 (1994) 397–402.
[16] M.T. Galceran, L. Puignou, M. Diez, J. Chromatogr. A 732 (1996) 167–174.
[17] M. Biesaga, M. Kwiatkowska, M. Trojanowicz, J. Chromatogr. A 777 (1997) 375–381.
[18] P. Kuban, B. Karlberg, Anal. Chem. 69 (1997) 1169–1173.
[19] H. Parvez et al. (Eds), in: Progress in HPLC-HPCE, Vol. 5, H. Parvez, P. Caudy, S. Parvez, P. Roland-Gosselin (Eds.), Capillary electrophoresis in biotechnology and environmental analysis, VSP BV.
[20] M. Harrold, M. Wojtusik, J. Riviello, P. Henson, J. Chromatogr. 640 (1993) 463–471.
[21] P. Doble, M. Macka, P. Haddad, Electrophoresis 19 (1998) 2257–2261.