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Separation and determination of sorbitol and xylitol in multi-component pharmaceutical formulations by capillary isotachophoresis

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Abstract

Pharmaceutically important polyhydric alcohols sorbitol (SO) and xylitol (XY) are efficiently separated and determined by analytical capillary isotachophoresis (ITP) with conductometric detection. The on-column complex-formation equilibria between the polyols and boric acid are utilized—the terminating borate ion acts as the complexing agent. The ITP operational system used consists of 10 mM HCl + 20 mM imidazole (LE, pH 7.0) and 20 mM boric acid (TE, pH 8. 0). The effective mobilities of the borated SO and XY are 8.3×10^{-9} m^2 V^{-1} s^{-1} and 7.4×10^{-9} m^2 V^{-1} s^{-1}, respectively. The ITP analysis is performed with the driving and detection currents of 50 μA (for 700 s) and 20 μA, respectively. The calibration graphs are rectilinear in the range 25–250 mg l^{-1} of SO and 50 to 500 mg l^{-1} of XY. The method is applied to the simultaneous assay of SO and XY in three mass-produced multi-component infusion solutions. Favourable values of the method validation parameters obtained confirm the suitability of the proposed ITP method for the quality control of pharmaceuticals.

Keywords: Sorbitol; Xylitol; Polyols; Infusion solutions; Pharmaceuticals; Isotachophoresis

1. Introduction

The polyols sorbitol (SO) and xylitol (XY) are utilized as major components of mass-produced parenteral preparations (infusion solutions) that are indicated as osmotic diuretics in the prevention and therapy of renal failure, in shock therapy and in the therapy of various intoxications. They are also used as a dietary additive in liver failure, for the treatment of the defects in water and mineral balance and in the therapy of some oedemas.

The United States Pharmacopoeia describes an HPLC method with a refractometric detection carried out at 30 ± 2°C for the identification and determination of pharmacopoeial polyhydric alcohols as bulk substances and as active ingredients in injections. It must be noted that this method, using a 30-cm × 7.8-mm column with water as

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mobile phase (flow rate, 0.2 ml min^{-1}) is of relatively low sensitivity and rather time consuming [1]. The British Pharmacopoeia [2] and Czechoslovak Pharmacopoeia [3] use iodimetric back-titration after oxidation of the hexitols with periodate. This method is also time consuming and cannot differentiate between various polyols if present in mixtures.

A number of instrumental analytical methods, such as high-performance liquid chromatography [4–7], flow injection analysis with amperometric detection [8] or capillary electrophoresis (CE) with laser-based interference refractive index detector [9] or CE in an arsenite buffer [10] have been applied to the analyses of selected polyhydric alcohols in recent years.

In our previous paper we reported on experimental conditions for the ITP determination of SO or mannitol in pharmaceutical formulations [11]. The operational electrolyte system consisted of 10 mM HCl + 20 mM imidazole (pH 7.1) as the leading electrolyte and 20 mM boric acid (pH ≈ 8) as the terminating electrolyte. In the present work the electrolyte system of similar parameters was used. The electroneutral polyols were converted into negatively charged B(III) complexes during the analysis and the derivatives thus obtained could be separated by ITP. This basic strategy for analyses of neutral polyhydric alcohols has been used in our present paper aimed to the develop of the selective method for the simultaneous determination of SO and XY in infusion solutions.

Generally, the reaction proceeding between boric acid and polyhydroxy substances to form borated polyols has been exploited earlier for detecting polyols or for titrimetric determination of boric acid as well as for a recently developed CZE method for the separation and detection of neutral carbohydrates [10,12,13].

2. Experimental

2.1. Materials

Standard of D-sorbitol (SO, Sanitas, Prague, Czech Republic) was of analytical grade and xylitol (XY, Sigma Aldrich, Prague, Czech Republic) was of quality complying with PhBs 4 [4]. All other chemicals were of analytical grade; they were obtained from Sigma–Aldrich and used as received. High-purity Millipore water was used throughout. All solutions were degassed by sonication (Tesson 1 ultrasonic bath, Tesla Prague, Czech Republic). Commercial infusion solutions were purchased from Infusia Hořátev, Czech Republic.

2.2. Instrumentation and isotachophoretic conditions

2.2.1. Apparatus

Isotachophoretic analyses were carried out with use of a ZKI 01 ITP analyser (Spišská Nová Ves, Slovak Republic) operated in the single-column mode. The analyser was equipped with a 30-μl sampling valve, a 120 × 0.3-mm (I.D.) analytical capillary made of fluorinated ethylene–propylene (FEP) copolymer, and a conductivity detector linked to a TZ 4600 chart recorder (Laboratorní přístroje, Prague, Czech Republic). Quantitative data were obtained from the length of the isotachophoretic zones, evaluated by manual processing of the first derivative of the conductivity signal versus the time of analysis, recorded at a suitable chart speed.

During the separation the TE (terminator) was protected from the atmospheric CO_2 by an adaptor packed with NaOH–asbestos mounted on the top of the TE chamber.

The pH values of the electrolytes were measured with an OP 211/1 pH-meter (Radelkis, Hungary) and a combined glass–SCE electrode.

2.3. Isotachophoretic procedures

2.3.1. Determination of effective mobilities ($\bar{u}$); calibration graphs

The determination of the effective mobilities of the analytes was carried out with aqueous 0.2 mM SO and 0.2 mM XY, 0.1 mM picric acid being utilized as the internal mobility standard. The driving and detection currents were 50 μA (700 s) and 20 μA, respectively. The effective mobilities of SO and XY were calculated from the values of

relative zone heights using the relation: $\bar{u} = 52.4/(h_{rel} + 0.66)$ (10^{-9} m^2 V^{-1} s^{-1}) (see Ref. [14]).

The leading electrolyte (LE) was a buffer solution of imidazole · HCl + imidazole (c (Cl^-) = 10 mmol l^{-1}, pH 7.04), containing 0.05% of poly(vinyl alcohol) as an additive. The terminating electrolyte (TE) was 20 mM boric acid treated with $Ba(OH)_2$ to adjust the pH ≈ 8.

The separation capacity of SO and XY was examined with three test solutions containing equimolar mixtures of the analytes with overall concentrations 3, 4 and 5 mmol l^{-1} that exceeded the separation capacity of the ITP column.

Aqueous working calibration solutions were prepared by appropriately diluting the stock standard solutions (500 mg l^{-1}) to obtain final calibration concentrations of 25–250 mg l^{-1} of SO, 50–500 mg l^{-1} of XY; they were analysed in triplicate at each concentration level.

2.3.2. Analysis of pharmaceutical preparations

Commercial infusion solutions were diluted with water to adjust appropriate concentration of the analyte falling within the calibration range (~ 100 mg l^{-1}). The content of SO and XY in a dosage form was calculated according to the regression equation taking into account the sample dilution.

2.3.2.1. Accuracy of the ITP method. In order to confirm accuracy of the ITP results, diluted infusion solutions (containing approximately 100 mg l^{-1} of the analytes) were analysed by the ITP directly and then again after the addition of known amounts (50.0 mg l^{-1}) of SO and XY standards [16]; the recoveries of the added amounts of SO and XY standards were calculated. Control iodimetric determinations were carried out following the standard pharmacopoeial method [3].

3. Results and discussion

3.1. Optimisation of ITP procedure

In the given ITP operational system (see Section 2) the values of effective electrophoretic mobilities, $\bar{u} = 8.3 \times 10^{-9}$ m^2 V^{-1} s^{-1} (for the borate–sorbitol complex) and $\bar{u} = 7.4 \times 10^{-9}$ m^2 V^{-1} s^{-1} (for the borate–xylitol complex) were determined. As shown in Fig. 1 the difference in the mobilities enables distinct separation of SO and XY under the optimum conditions. Therefore the proposed method could be used for the analysis of composite pharmaceuticals containing both SO and XY. To substantiate applicability of the ITP separation to the analysis of real samples the optimisation of ITP procedure also involved the definition of the conditions of complete separation of SO and XY, i.e. the evaluation of their separability as maximum amount of the equimolar mixture of SO and XY injected at which their mixed zone would not yet be formed. This was accomplished by analysing several test solutions of equimolar SO–XY mixtures at concentrations exceeding the separation capacity of this system (cf. Ref. [15]). In this experiment the quantitative parameter measured was the electric charge Q.

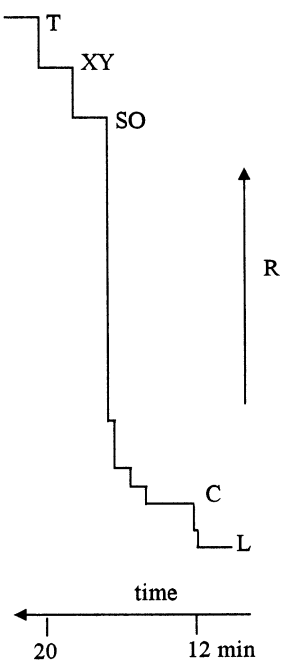

Fig. 1. Isotachopherogram of Nutramin NEO SX 4%. SO, sorbitol; XY, xylitol; C, hydrogen carbonate. Operational system: HCl/imidazole, pH 7.0 (L), and borate (T). The lower part of the ITP record involves zones of unidentified anionic ingredients of the tested preparation (amino acids)

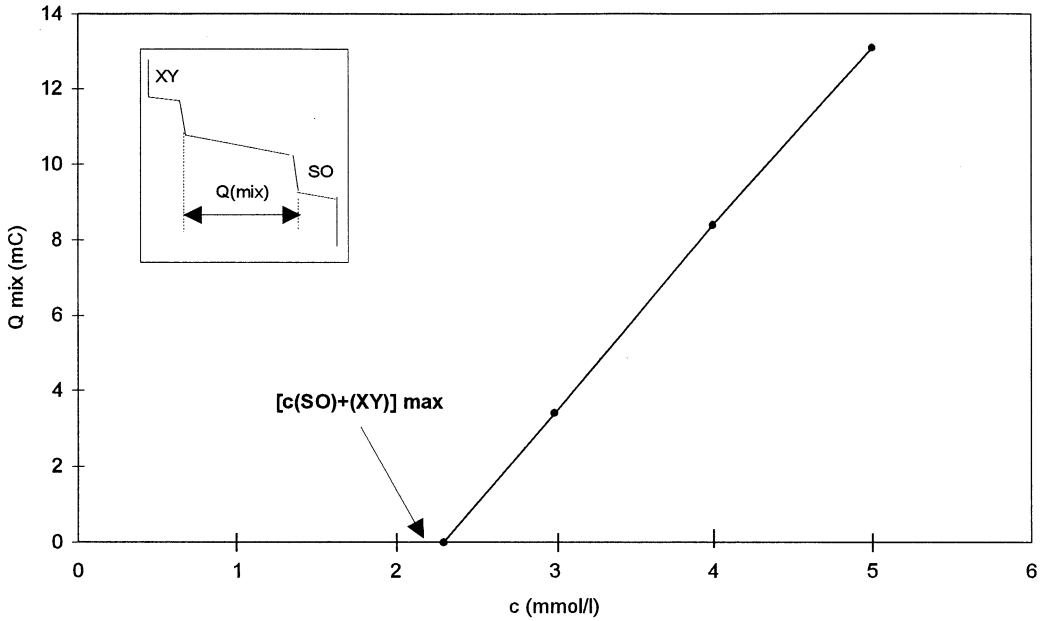

Fig. 2. Graphic evaluation of maximum separable amount for an equimolar mixture of sorbitol and xylitol

The hold-up of the column was 44.0 ± 1.3 mC. The zones of pure analytes (SO and XY) and a mixed zone of SO + XY (Q_{mix}) can be clearly seen in Fig. 2. The relation between Q_{mix} and the sample injected is rectilinear. The straight line intersects the x-axis at $Q_{mix} = 0$; the intersection denotes the limiting concentration of the mixture at which perfect separation of the analytes without the formation of the mixed zone is achieved ($c = 2.3$ mmol l^{-1} at sample volume of 30 ml). This corresponds to concentrations of 417 mg l^{-1} (SO), or 348 mg l^{-1} (XY). Hence the concentrations of the samples should be adjusted before the ITP analysis in such a way that they would not exceed the indicated separation capacity values.

3.2. Calibration graphs

The calibration dependences of SO and XY in the ranges of $s = 25$–250 mg l^{-1} and 50–500 mg l^{-1}, respectively, were examined. A linear regression evaluation of the ITP zone length l (mm) versus analyte concentration c (mg l^{-1}) relationship gave quotations $l = 0.17488c - 0.2556$ for SO and $l = 0.21542c - 3.0131$ for XY, with the correlation coefficients of 0.99876 and 0.99836, respectively (number of calibration points = 6). The low values of the intercepts and the high values of the correlation coefficients are indicators of analytical stability of the ITP zones and rectilinearity of the calibration curves, respectively. Good precision of the ITP method is characterised by favourable relative standard deviation values R.S.D. = 1.26% (SO) and 1.44% (XY) of the results obtained at analyte concentrations of 150 mg l^{-1} ($n = 6$).

3.3. Determination of polyols in infusion solutions, the accuracy of the ITP method

The proposed method was applied to the simultaneous separation and determination of SO and XY in composite infusion solutions. Typical isotachopherogram of a real sample indicating the absence of any interfering zones is shown in Fig. 1. Table 1 shows the results of the ITP assay of SO and XY in three preparations; the results obtained by the ITP method are accompanied by those showing ITP recovery of standard additions of SO and XY to the original samples [17]. The recovery results (98.8–103.4%) confirm that the

Table 1
Determination of sorbitol and xylitol in comercial infusion solutions

Preparation	Polyol	Nominalcontent (g l^{-1})	Found[a] (iodimetry), $n=6$	Found[a] (ITP), $n=6$	Added (g l^{-1})	Found[b] (ITP)	% R
Nutramin NEO SX 8%	SO	50	96.02 ± 0.52	52.20 ± 1.23	50.0	51.20 ± 1.27	102.4
	XY	50		49.35 ± 1.52	50.0	51.70 ± 1.36	103.4
Nutramin NEO SX 4%	SO	50	95.10 ± 0.83	52.20 ± 1.25	50.0	51.30 ± 1.40	102.6
	XY	50		49.00 ± 1.20	50.0	49.40 ± 1.22	98.8
NUTRAMIN U	SO	25	48.70 ± 1.11	26.25 ± 1.13	50.0	51.10 ± 1.32	102.2
	XY	25		24.97 ± 1.15	50.0	51.25 ± 1.50	102.5

[a] In the original sample.
[b] Of the added amount; recovery = $(100 \times \text{Found}^b/\text{Added})$.

ITP method gives accurate results and that it can be used for simultaneous determination of SO and XY content in commercial infusion solutions. The reproducibility of the ITP results is characterised by the %R.S.D. values ranging between 1.1 and 1.5% ($n=6$).

It is difficult to compare statistically the ITP results obtained with the composite infusion solutions with those of the pharmacopoeial iodimetric method [3] by, e.g. Student's t-test, since the latter method gives only the sum of the polyol content. Therefore, in Table 1, we include the values for the overall polyol content found by iodometry and expressed as the amount of sorbitol.

4. Conclusions

The content of selected polyhydric alcohols in commercial infusion solutions was quantitated by the ITP method described above. The proposed method is sufficiently simple, sensitive and selective enough to be applied to the simultaneous determination of sorbitol and xylitol in composite infusion solutions. The preparation of samples for the ITP analysis involves just dilution with water to achieve an appropriate concentration level of the analytes in the test solution falling within the calibration range. The ITP analysis is carried out in purely aqueous medium and therefore it can be considered as ecologically and economically friendly. The ITP method gives relatively reproducible and accurate results (see the data in Table 1). Hence it may be recommended as an interesting and cost-effective alternative to other separation techniques for the quality control of multicomponent infusion solutions in pharmaceutical analysis. Moreover, the proposed ITP method can successfully substitute nonselective chemical or instrumental techniques such as titrimetry or spectrophotometric methods that are not capable of determining individual components in mixtures of polyhydric alcohols.

Acknowledgements

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References

[1] United States Pharmacopeia (USP), 22nd Revision, The National Formulary 17th ed., US Pharmacopeial Convention, Rockville, MD, 1990, p. 1985.
[2] British Pharmacopoeia (BP), HM Stationery Office, London, 1993, p. 624.
[3] Czechoslovak Pharmacopoeia (PbBs), 4th ed., vol. III, Avicenum, Prague, 1987, pp. 847, 941.
[4] A. Zaton, G. Quindos, J. Ponton, J. Chromatogr. 525 (1990) 169–175.
[5] C. Vicente, J.L. Mateos, M.M. Pedrosa, M.E. Legaz, J. Chromatogr. 553 (1991) 271–283.
[6] T. Soga, Y. Inoue, K. Yamaguchi, J. Chromatogr. 625 (1992) 151–155.

[7] J. Oehlke, M. Brudel, I.E. Blasig, J. Chromatogr. B 655 (1994) 105–111.
[8] T.R.I. Cataldi, D. Centoze, Anal. Chim. Acta 43 (1995) 307–312.
[9] J.C. Ren, Y. Z. Deng, J.K. Cheng, Fenxi-Huaxue, 21 (1993) 1374-1377; Anal. Abstr. (1993) 5606E139.
[10] J.C. Ren, Y.Z. Deng, J.K. Cheng, Sepu 13 (1995) 244-246; Anal. Abstr. (1995) 5712F065.
[11] M. Pospísilová, M. Polásek, J. Procházka, J. Chromatogr. A 772 (1997) 277–282.
[12] S. Honda, J. Chromatogr. A 720 (1996) 337–351.
[13] A. Paulus, A. Klockow, J. Chromatogr. A 720 (1996) 353–376.
[14] V. Jokl, J. Pospíchalová, M. Polásek, Electrophoresis (Weinheim) 7 (1986) 433–435.
[15] P. Gebauer, P. Boèek, J. Chromatogr. 320 (1985) 49–65.
[16] State Inst. Drug Control Information Bulletin, Skarnitzl Foundation Publishers, Prague, no. 11, 1991, pp. 1–5.
[17] L. Huber, Good Laboratory Practice, Hewlett-Packard Company, Hewlett-Packard publication number 12-5091-6259G (1993) p. 45.