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Journal of Chromatography A, 772 (1997) 277–282

JOURNAL OF CHROMATOGRAPHY A

Separation and determination of pharmaceutically important polyols in dosage forms by capillary isotachophoresis

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Abstract

Capillary isotachophoresis (ITP) with conductometric detection has been used for separating polyols such as mannitol, sorbitol, dulcitol and xylitol. Since the polyols behave as extremely weak acids in aqueous medium, the complex-formation equilibria between boric acid and the analytes was employed to convert the neutral analytes into ionic species. The characteristic feature of this approach is the use borate, which functioned both as the complexing agent and the terminating ion. The ITP electrolyte system, optimised with respect to the sensitivity of the ITP determination and the quality of separation, consisted of 10 m*M* HCl+20 m*M* imidazole (pH 7.1) containing 0.05% poly(vinylalcohol) as the leading electrolyte and 20 m*M* boric acid (pH 8.1) as the terminating electrolyte. The driving and detection currents were 50 μA (for 630 s) and 20 μA, respectively. The effective mobilities of the borate–polyol complexes were determined; efficient separation of mannitol–sorbitol, sorbitol–xylitol and sorbitol–dulcitol mixtures was achieved. The calibration graphs were rectilinear (r=0.9995–0.9999) in the ranges 10–200 mg l^{-1} of sorbitol or mannitol. The relative standard deviations were 1.4 to 1.8% (n=6) when determining 100 or 200 g l^{-1} of mannitol and/or 50 to 200 g l^{-1} of sorbitol in mass-produced infusion solutions. A single analysis took about 20 min. The results obtained by the ITP method were in good agreement with those of the standard pharmacopoeial iodimetric method.

Keywords: Isotachophoresis; Pharmaceutical analysis; Polyols

1. Introduction

The hexitols mannitol and sorbitol and pentitol xylitol are pharmaceutically important non-ionic osmotic diuretics. Infusion solutions of such polyols find wide use in the therapy and prevention of renal failure of different aetiology. They can also be found as components of composite parenteral nutrition solutions that are indicated in various pathological states. Another biologically important hexitol is galactitol (dulcitol), which is, like the other hexitols, often used for its taste as a dietary sweetener additive in the manufacture of food products.

Aliphatic polyhydroxy compounds can be determined by chemical methods, such as, e.g., iodimetric back titration after oxidation of the analyte with periodate; although the method is official [1], it is rather time-consuming and relatively non-selective. The tendency of polyols to form chelate complexes with some central ions such as B(III), Ge (IV) or Mo(VI) is of analytical importance since the complex formation is accompanied by the liberation of protons from the polyol molecule that are normally not split off in aqueous medium. The pH changes occurring due to complex formation have been utilized earlier in the potentiometric [2,3] or spectrophotometric determination of mannitol and sorbitol [4]. Various alternative instrumental methods includ-

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PII S0021-9673(96)00862-X

ing liquid chromatography [5], HPLC [6,7], gas chromatography [8,9], capillary electrophoresis with laser-based interference refractive index detection [10] and amperometric determination of alditols using a flow injection analysis system with a chemically modified indicator electrode [11] have also been reported.

In this paper, a simple, accurate and relatively sensitive and rapid method for isotachophoretic separation and determination of the cited polyols has been devised; its applicability to the analysis of some pharmaceutical preparations has been demonstrated.

2. Experimental

2.1. Instrumentation

Isotachophoretic analyses were carried out using a ZKI 01 ITP analyser (Spišská Nová Ves, Slovak Republic) in the single-column mode. The analyser was equipped with a 30-μl sampling valve, a 120-mm×0.3 mm I.D. analytical capillary, made of a fluorinated ethylene–propylene (FEP) copolymer, and a conductivity detector. The detector signal was recorded by 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 atmospheric CO_2 by using 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 (Radelkis, Hungary) pH-meter and a combined glass–SCE electrode.

2.2. Chemicals

Pure standards of D-mannitol (MA; Lachema, Brno, Czech Republic), D-sorbitol (SO; Sanitas, Prague, Czech Republic), dulcitol (galactitol; DU) and xylitol (XY; Sigma Aldrich, Prague, Czech Republic) were of quality complying with PhBs 4 [1] for SO and MA or of analytical grade. Distilled and demineralised water was used throughout for the preparation of the solutions. All solutions were filtered through a 0.85-μm filter (Synpor, Prague, Czech Republic) and were degassed by sonication (Tesson 1 ultrasonic bath, Tesla, Prague, Czech Republic). Commercial infusion solutions containing 50, 100 and 200 g l^{-1} of SO or 100 and 200 g l^{-1} of MA were purchased from Medicamenta (Vysoké Mýto, Czech Republic) or Praxipharma (Prague, Czech Republic). All other chemicals were of analytical grade.

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 m*M* SO and 0.4 m*M* MA, XY or GA, with 0.1 m*M* picric acid being used as the internal mobility standard. The driving and detection currents were 50 μA (630 s) and 20 μA, respectively. The effective mobilities of drugs under study were calculated from the values of relative zone heights [12].

Aqueous working standard solutions for calibration purposes were prepared by appropriately diluting the stock standard solutions (200 mg l^{-1} of MA or 500 mg l^{-1} of SO) to obtain final calibration concentrations of 10–200 mg l^{-1} of an analyte.

2.3.2. Analysis of pharmaceutical preparations

Infusion solutions were diluted with water to adjust the approximate concentration of the analyte to fall within the calibration range. The content of a drug in a dosage form was calculated according to the regression equation, taking dilution of the sample into account.

Control iodimetric determinations were carried out following a standard pharmacopoeial method [1].

3. Results and discussion

3.1. Selection of a suitable electrolyte system and principal ITP characteristics of the analytes

As follows from the acid–base properties of the polyols under study, the analytes are not ionized,

Table 1
Optimised aqueous operational system used for ITP of polyols

	Anion	Counter-ion	pH	Additive
Leading electrolyte	10 m*M* Cl^-	20 m*M* IMI	7.1	0.05% (w/v) PVA
Terminating electrolyte	20 m*M* borate		~8.1[a]	

Note: IMI = imidazole; PVA = poly(vinylalcohol);
[a] adjusted with 0.1 *M* $Ba(OH)_2$.

even at extremely high pH values, in aqueous solutions. Therefore, direct ITP analysis of them is impossible. To facilitate the electro-migration of the polyols, the strategy based on their conversion to anionic species by complex formation with B(III) as the central ion has been adopted. The hydroxy groups of the polyols react rapidly with borate to give anionic complexes that could presumably be separated by ITP. Generally, the reactions proceeding between polyols (L) and boric acid can be schematically expressed by Eqs. (1,2) in solutions with low and high polyol–boric acid concentration ratios, respectively (cf. [13]):

$$B(OH)_3 + L \leftrightarrow H^+ + [B(OH)_2(H_{-2}L)]^- + H_2O \quad (1)$$

$$B(OH)_3 + 2L \leftrightarrow H^+ + [B(H_{-2}L)_2]^- + 3H_2O \quad (2)$$

These reactions have been commonly used for detecting polyols and for titrimetric determination of boric acid [2].

In this paper, the complex-forming agent, i.e. boric acid, served as the TE and, thus, in situ conversion of the neutral analytes to ionic species took place only during the ITP analysis. The ITP operational system has been optimised with respect to the quality of separation and the sensitivity of the ITP determination. Various operational systems with Cl^- as the leading ion have been tested. The optimisation involved critical selection of the kind (histidine, imidazole, Tris and dimethylaminoethanol) and the concentration of the counter-ion and, consequently, the pH of the leading electrolyte (LE), which varied over the range 6.1 to 9.0. The optimised parameters of the operational electrolyte system are shown in Table 1. With the optimised composition of the LE (involving imidazolium as the counter-ion), the maximum buffering capacity of the LE and hence the stability of the chemical equilibria in the steady-state is guaranteed. The selected pH of the system is sufficiently high to promote the formation of the borate–polyol complexes. Since the concentration of the TE (borate) relative to analytes exceeds a ratio of twenty, the reaction equilibrium described by Eq. (1) can be expected to take place during the ITP separation; moreover, the mobility of the TE is low enough to allow for the separation of low-mobility analytes of weakly acidic character. The ITP analysis takes about 20 min if the driving current–time regime shown in Section 2 is followed.

Effective electrophoretic mobilities, $\bar{u}$, of polyol–borate complexes, determined with the use of picric acid as the internal standard, are presented in Table 2; these values have not been reported earlier. The differences among the observed mobilities allow reliable separation of MA–SO, SO–XY and SO–DU mixtures — see also the isotachopherograms in Fig. 1. In the lower part of these isotachopherograms, the zones of carbonate (C) and an unidentified impurity can be observed. From the standpoint of quantitative pharmaceutical analysis, the separation of MA and SO by ITP is not of great importance since, in real dosage forms, the given drugs usually occur separately, however, the difference in their mobilities might be used for identification purposes. Perfect separation of SO and XY in the electrolyte system employed is of practical importance since, in some

Table 2
Effective mobilities of polyol–borate complexes

Polyol	$\bar{u} \times 10^9 \pm$R.S.D.[a] ($m^2\ V^{-1}\ s^{-1}$)
MA	7.41±1.22
SO	8.15±1.37
DU	7.38±1.12
XY	7.11±1.11

For the parameters of the electrolyte system, see Table 1; [a] $n = 3$; $\bar{u}_T = 6.66 \cdot 10^{-9}$ $m^2\ V^{-1}\ s^{-1}$ (R.S.D. = 1.35%).

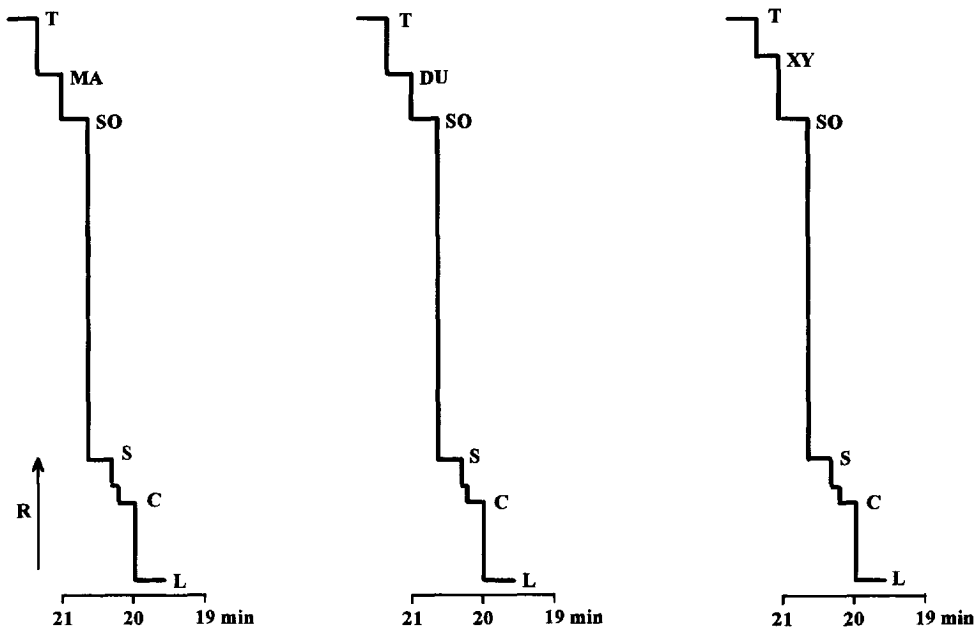

Fig. 1. Separation (from left to right) of mannitol (MA), sorbitol (SO), xylitol (XY) and dulcitol (DU) as the borate complexes formed on-column. Operational system: HCl–imidazole, pH 7.1 (LE) and boric acid (TE); S = picric acid as the mobility standard; C = hydrogen carbonate.

commercial composite infusion solutions, both SO and XY are present together.

3.2. Calibration graphs

The applicability of the devised ITP method in pharmaceutical analysis has been exemplified by the determination of MA and/or SO in commercial infusion solutions. For the determination of the individual hexitols, the method of external standardization has been used. The calibration dependence of MA and SO standards $l = f(c)$ (where l stands for the ITP zone length) were examined at concentration ranges of 10–200 mg l^{-1} of an analyte and evaluated by linear regression. The linear regression parameters of the calibration lines are summarized in Table 3.

The low values of the intercepts and the high values of the correlation coefficients are positive signs of the analytical stability of the zones and of rectilinearity of the calibration curves, respectively. The results for replicate analyses of prepared samples of 100 mg l^{-1} of MA ($n = 6$) and 50 mg l^{-1} of SO ($n = 6$) had relative standard deviations (R.S.D.s) of 0.30 and 1.22%, respectively, and characterize good reproducibility of the ITP method.

3.3. Determination of drugs in pharmaceutical formulations

The determination of mannitol and/or sorbitol in pharmacopoeial infusion solutions [1] has been carried out. In all instances, single-component hexitol preparations were analysed. As mentioned above, MA and SO do not occur together in real dosage

Table 3
Linear regression calibration parameters[a] of hexitols ($c = 10$–200 mg l^{-1})

Drug	a	$s(a)$	b	$s(b)$	r
Mannitol	0.08098	0.00025	0.08789	0.02809	0.9999
Sorbitol	0.08255	0.00134	0.47248	0.15102	0.9995

[a] $l = ac + b$, where l = ITP zone length (mm), c = analyte concentration (mg l^{-1}), a = slope, $s(a)$ = standard deviation of the slope, b = intercept, $s(b)$ = standard deviation of the intercept, r = correlation coefficient and n, the number of calibration points = 6.

Table 4
Isotachophoretic determination of mannitol and sorbitol in pharmacopoeial infusion solutions

Formulation	Hexitol nominal content	Content (%±R.S.D.)		
		ITP method ($g\ l^{-1}$)	Standard method ($n=6$)	Student's t-test[a] ($n=6$)
Infusio mannitoli 10	100	103.83±1.42	102.70±2.43	0.871
Infusio mannitoli 20	200	102.68±1.45	100.60±1.54	2.170
Infusio sorbitoli 5	50	98.45±1.75	101.01±1.92	2.208
Infusio sorbitoli 10	100	97.89±1.45	99.56±1.79	1.640
Infusio sorbitoli 20	200	96.72±1.51	98.43±2.25	1.444

[a] 95% confidence level; $t_c=2.228$ ($\nu=2n-2$); $n=6$.

forms, but some non-pharmacopoeial infusion solutions that contain both sorbitol and xylitol are commercially available. Although we have shown that SO and XY are readily separated by ITP, the analyses of multi-component polyol preparations will be dealt with in our forthcoming communication.

Because of the simple matrix of the infusion solutions under study, the sample preparation for analysis was straightforward. The sample was just diluted with water to achieve an appropriate concentration that fell within the ITP calibration range. The results obtained by the ITP analyses of infusion solutions, together with those of the reference pharmacopoeial titrimetric method [1] and appropriate inter-assay validation [14], are summarised in Table 4. It can be clearly seen that the content of MA or SO found by the ITP method is in agreement with the nominal content of the active ingredient in the infusion solutions. The preparations comply with the PhBS 4 [1] demanding 95 to 105% of the drug. No statistically significant difference is observed between the results obtained with the ITP method and the reference method.

4. Conclusions

The results presented in this work corroborate the fact that the anionic capillary ITP method is a suitable tool for separating selected pharmaceutically important electro-neutral polyols, namely mannitol, sorbitol, galactitol and xylitol. The method is based on an on-column complexation of the analytes (playing the role of the ligand) with an excess of borate as the central ion (acting as the terminating ion as well), resulting in the formation of electromigrating anionic species. The work is focused on the application of ITP to the quantitative analysis of pharmacopoeial infusion solutions. Most of the previous papers concerning the analysis of polyols dealt either with quantitative analysis of such compounds as bulk substances by rather unselective non-separation techniques or with the analysis of model mixtures using separation techniques within the framework of inorganic and organic analysis. Although some previously published methods are more sensitive than the proposed ITP method, this issue is not of key importance when analysing commercially available pharmaceuticals that contain, as a rule, relatively high amounts of the polyols. Although the proposed ITP method requires derivatisation of the analytes, the method is acceptably time efficient, due to the on-column technique employed; a single analysis takes over 20 min (including the time involved in sample preparation). Moreover, the advantage of ITP is that the migration medium employed is purely aqueous, without any of the organic solvents that are needed in most HPLC analyses. The precision of the method, expressed as the R.S.D., was 1.4–1.8% (6 replicates) when analysing five commercial preparations and for all five preparations analysed, no significant differences were found between the results obtained by ITP method and the pharmacopoeial iodimetric method for the same batches, at the 95% confidence level (Student t-test, cf. Table 4). Therefore, the ITP method can be recommended for analytical evaluation of commercial infusion solutions containing mannitol or sorbitol as the active ingredient.

Acknowledgments

This work was supported by the Charles University Grant Agency, grant No. 269/94.

References

[1] Czechoslovak Pharmacopoeia (PhBs), Vol. II, Avicenum, Prague, 4th ed., 1987, pp. 511 and 847.
[2] M. Mikešová and M. Bartušek, Scripta Fac. Sci. Nat. Univ. Purk. Brun., 9 (1979) 13.
[3] M. Bartušek and V. Zdražilová, Scripta Fac. Sci. Nat. Univ. Purk. Brun., 15 (1985) 305.
[4] M. Bartušek and V. Zdražilová, Scripta Fac. Sci. Nat. Univ. Purk. Brun., 15 (1985) 331.
[5] H. Caruel, L. Rigal and A. Gaset, J. Chromatogr., 558 (1991) 89.
[6] United States Pharmacopoeia, 22nd Revision, US Pharmacopoeial Convention, Rockville, MD, 1990, pp. 802 and 1985.
[7] Q.B. Luo, Fenxi Ceshi Xuebao, 12 (1993) 73; Anal. Abstr. 5607G004.
[8] M. Cheng, A. Ren and X. Xie, Yiyao Gongye, 19 (1988) 73; Anal. Abstr. 5010E100.
[9] M. Cai, G. Chen and P. Li, Sepu, 6 (1988) 376; Anal. Abstr. 5108D113.
[10] J.C. Ren, Y.Z. Deng and J.K. Cheng, Fenxi Huaxue, 21 (1993) 1374; Anal. Abstr. 5606E139.
[11] T.R.I. Cataldi and D. Centonze, Anal. Chim. Acta, 307 (1995) 43.
[12] V. Jokl, J. Pospíchalová and M. Polášek, Chem. List., 80 (1986) 1305.
[13] A.E. Martell and R.M. Smith, Critical Stability Constants, Vol. 3, Plenum Press, New York, 1977, p. 270.
[14] K. Eckschlager, I. Horsák and Z. Kodejš, Evaluation of Analytical Results and Methods, SNTL, Prague, 1980, p. 44.