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Determination of Isosorbide Nitrate and Its Analogues in Pharmaceuticals by High-Performance Liquid Chromatography

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ABSTRACT

A reversed-phase column liquid chromatographic method was developed for analysis of isosorbide dinitrate (ISDN), isosorbide 5-mononitrate (IS5MN) and isosorbide 2-mononitrate (IS2MN) and its pharmaceuticals. Chromatography is performed to Bondclone ODS column using a mobile phase consisting of acetonitrile-PIC B6 buffer, pH 4.75 (13:87) with ultraviolet wavelength at 220 nm. The linearity calibration range was 0.05 mg/ml to 2.5 mg/ml (r=0.9999), and recoveries were generally greater than 98.0%

Key words : Isosorbide dinitrate, Isosorbide 5-mononitrate, Isosrobide 2-mononitrate, HPLC.

INTRODUCTION

Isosorbide dinitrate (ISDN), Isosorbide 5-mononitrate (IS5MN) and Isosorbide 2-mononitrate (IS2MN), are members of a group of vasodilator drugs that are used in the treatment of angina pectoris and ischemia of skeletal muscles. The last two are metabolites of ISDN and are less active than parent drug[1 2]. Since isosorbide nitrate esters have slow onset of action and their duration are longer than that of nitroglycerin, they are beneficial to the prevention of heart attack but not in the management of acute attack[1].

ISDN has been determined by color development with phenodisulfonic acid[3-4], IR spectrophotometry[5 6], Polargraphic method[7-9], and NMR[10], because those methods were unable to be distinguished among the decomposition products, Gas-liquid chromatography[11-12] coupled with electron capture detection, is most commonly used. Electron capture, though sensitive, suffers from lack of reproducibility, detection contamination, special techniques must be employed to maintain linearity of the detector response. Also, results in partial decomposition of ISDN are due to its thermal nature at elevated temperature. More recently high-performance liquid chromatographic (HPLC) methods have been described that for the analysis of the cardiovascular agents including ISDN[13-15].

The HPLC methods eliminate interference from the organic or inorganic nitrates and allow ISDN to be distinguished from its 2-mononitrate degradation product. The study of ISDN

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and its analogues represent a distinct challenge because of the lack of a UV chromophore in the molecule. Ultraviolet detection at low wavelengh limits the mobile phase modifier that can be used to enhance the selectivity and peak shape. Also, the use of low-pH mobile phase results in concerns about column life and method performance over a period of time. The current report describes methods for dealing with the above problems and provides an assay procedure for the determination of ISDN and its analogues in their pharmaceuticals and bulk drug.

MATERIALS AND METHODS

I . *Chromatographic apparatus*

The HPLC system consisted of a dual-piston pump and solvent delivery system (Waters model 600E), a tunable absorbance detector (Waters model 486 E), an autosampler with universal injector (Waters 700 Satellite WISP). The separation was performed on a 250 × 4.0 mm i.d. analytical column containing 10 um ODS (Phenomenex Bondclone C_{18}, 2320 W. 205th St., Torrance, CA 90501 U.S.A.). The chromatographic peaks were integrated (Waters, Data Module 745B) and recorded.

II. *Materials*

Acetonitrile and phosphoric acid were HPLC grade (E. Merck). Sodium hexanesulfonic acid was purchsed from Sigma Chemical Co. (P. O. Box 14508 St. Louis MO 63178 U.S.A., Lot. 119F5608). The internal standard, 4-aminoacetophenone was analytical grade (E. Merck, Darmstart, F. R. Germany).

The standard isosorbide dinitrate diluted in 60% lactose were purchased from Sigma Chemical Co. (P.O. Box 14508 St. Louis, MO 63178 U.S.A. Lot. 16F05341). It was extracted with methanol and filtered. The filtrate was evaporated to dryness under a current of nitrogen flow and the residue was assayed by the compendial method[3] and the potency was 99.6%.

Isosorbide 5-mononitrate was purchased from Tokyo Kasei Kogyo Co., LTD. (3-1-13, Nihonbashi-Honcho, Chuo-Ku, Tokyo 103 Japan), standard isosorbide 2-mononitrate was donated by M. Carlson. Various dosage forms or formulations in this study were obtained from the application process for production or free sale licences. All excipients used in synthetic formulations were reagent grade. Water was deionized HPLC grade was filtered by a 0.22 um membrane filter (Milli-Q SP Reagent Water System, Waters Co. LTD., 34 Maple St. Milford MA 01757 U.S.A.).

Solution A was prepared solvent composition of acetonitrile and water (6 : 4) was prepared for dissolving standard drug, synthetic mixtures and pharmarceuticals.

III. *Chromatographic condition*

A solution of 870 ml of 0.005 M sodium hexanesulfonic acid and 130 ml of acetonitrile was mixed and adjusted to pH 4.75 with phosphoric acid. The solution was filtered through a membrane of 0.45 um, degassed under a helium flow, and was used as the mobile phase for HPLC.

IV. *Internal standard*

An accurately weighed 200 mg of 4-aminoacetophenone was transfered to a 100-ml volumetric flask, and about 75 ml of solution A was added. The flask was placed in an ultrasonic bath for 3 min. and diluted to volume with solution A. The resulting solution was used as the internal standard solution.

V . *Standard solution*

Transfered an accurately weighed 100 mg of ISMN, IS5MN, IS2MN and about 75 ml of Solution A to a 100-ml volume flask. Placed the resulting mixture in an ultrasonic bath and shaked for 5 min.. A 10.0 ml of internal standard stock solution was added, and the solution was

added to volume with solution A. This standard solution contained 1.0 mg/ml of ISDN, 0.2 mg/ml of IS5MN, 0.2 mg/ml of IS2MN and 0.2 mg/ml of 4-aminoacetophenone, respectively.

VI. *Sample preparations*

Aliquots of a powder blended from 10 tablets of a commercial formulation, equivalent to 5.0 mg, 10 mg, 20 mg of ISDN, slurried with 30 ml of solution of acetonitrile-water (6:4), the resulting mixture was shaken well for 30 min., mixed with 10 ml of internal standard stock solution, and diluted with 60 ml of solution A. Transfered aliquots of a commercial product blended powder, equivalent to 20 mg of IS5MN was made up with same procedure stated above. Certain oral timed-released tablet required longer agitation to aid solution completely. A portion of all the sample solutions was passed through a centrifuge filter containing a 8-mm diameter, 0.45 um. porosity cellulose acetate prior to injection and analyzed.

VII. *Analysis*

The chromatographic conditions used for analysis were : flow rate, 1.5 ml/min.; detector, 220 nm, Att., 128; injection volume, 10 ul of standard preparation and the filtrate from the synthetic formulation sample and commercial product preparations; and the temperature is ambient.

Quantitative analysis was determined by comparing the peak area response ratios of ISDN, IS5MN and IS2MN to the 4-aminoacetophenone from a sample injection to the corresponding area ratio from a standard five injection, respectively.

VIII. *Recovery Study*

The accuracy of the current procedure was assessed by the use of synthetic formulations. Four placebo formulations were prepared representing a diluted bulk drug and various tablet dosage forms containing commonly used excipient materials. These mixtures were prepared by thoroughly blending the materials in a porcelain mortar. Replicate portion of the placebo formulations were fortified with ISDN and IS5MN at the 100 % level and carried through the procedure of analysis.

IX. *Linearity*

A calibration curve based on the peak response ratios of ISDN, IS5MN and IS2MN to the 4-aminoacetophenone internal standard versus ISDN, IS5MN and IS2MN concentrations was constructed to determine the linearity of the chromatographic response. Standard solutions containing 0.05, 0.1, 1, 2, 2.5 mg/ml of ISDN, IS5MN and IS2MN, each having an internal standard concentration of 0.1 mg/ml, were used.

RESULTS AND DISCUSSION

Linearity

Linearity of ISDN and its analogues were evaluated at low and high concentration ranges. Dilute standards of nitrate esters over a range of 0.05-2.5 mg/ml were verified by injection five solutions respectively. It provided a straight line

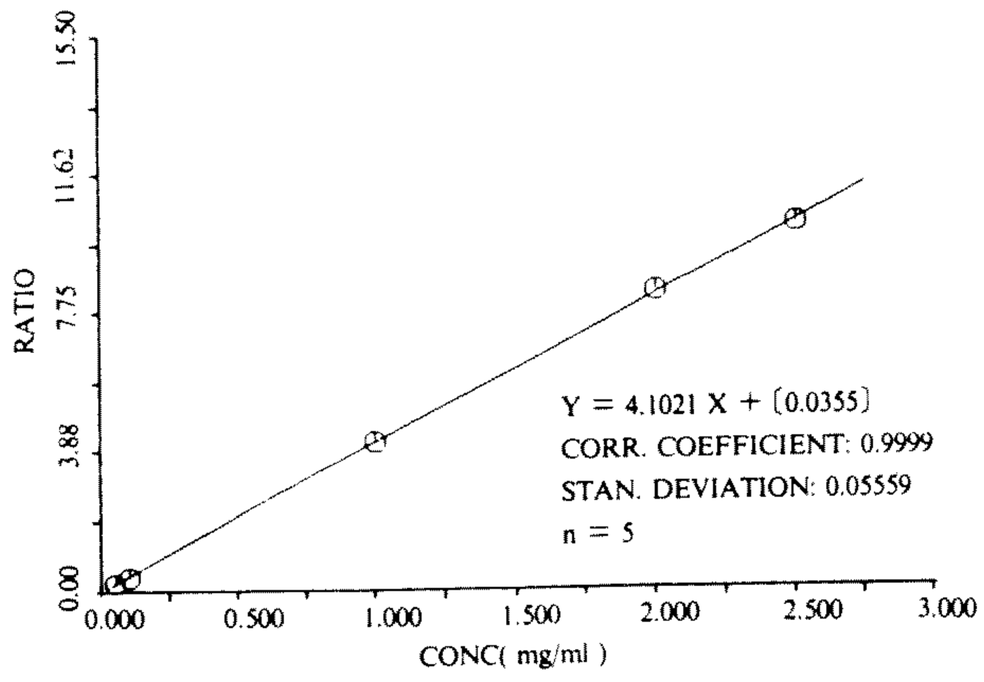

Figure 1. Standard curve for isosorbide 2-mononitrate. The points of curve are at 0.05, 0.1, 1.0, 2.0, and 2.5 mg/ml.

with good coefficient of determination for n=5 injections and good %RSD. As shown in Figure 1, 2, 3. γ=0.9999 [y = 4.1021 x + (0.0355)], and a RSD of 0.05% for IS2MN, γ=0.9999 [y = 4.2493 x + (-0.0250)] and RSD of 0.08% for IS5MN, γ= 0.9998 [y = 5.5759 x + (-0.0011)] and RSD of 0.48% for ISDN. From the data observed on the figures, γ>0.9998 and RSD < 0.5%, this demonstrated the linearity of response for standrard solution in the studied method.

Reproducibility of those nitrate esters for both within-day and the between-day assay were evaluated. The coefficient of variation at the amount of 10 μg, on the basis of peak area ratios for standard IS2MN, IS5MN, and ISDN six replicate injections in the within-day assay, IS2MN was from 0.1 to 0.5%, IS5MN was between 0.06 to 0.4% and ISDN was between 0.1 to 0.5%. The coefficient of variation in between-day assay (n=5) was 0.23 % at same amount. As expected, the within-day RSD in the range from 0.16 to 0.5% were much lower, because it was not affected by as many variables.

Recovery study

The recovery percentage for four synthetic mixtures were determined on the Table 1. is ranged from 99.0% to 100.8% and the C.V. value ranged from 0.08 to 0.13%. According to the configuration of IS5MN, it has electron withdrawing group at equatorial and oxygen atom at same plane, which is further distance than that IS2MN of same group at axial. Thereby, the adsorption of IS2MN is less than that of IS5MN, the C.V. value was smaller than IS5MN and ISDN, and eluated with short retention. The largest C.V. value for ISDN might be produced by prolonging heat during sample preparation and eluting slowly with 28.5 min due to higher polarity. Becuase the avaerage recovery was > 99.0% and C. V. value was < 0.13%, obviously, no interference due to the excipients could

Table 1. Recovery of IS5MN, IS2MN and ISDN from synthetic formulation

Formulation

		mg added	mg mean	% (n=5) recovery	% C. V.
1	ISDN	4	3.97	99.2	0.09
2	IS5MN	4	4.03	100.8	0.08
3	IS2MN	3	2.95	98.6	0.10
4	ISDN	5	4.92	98.4	0.13

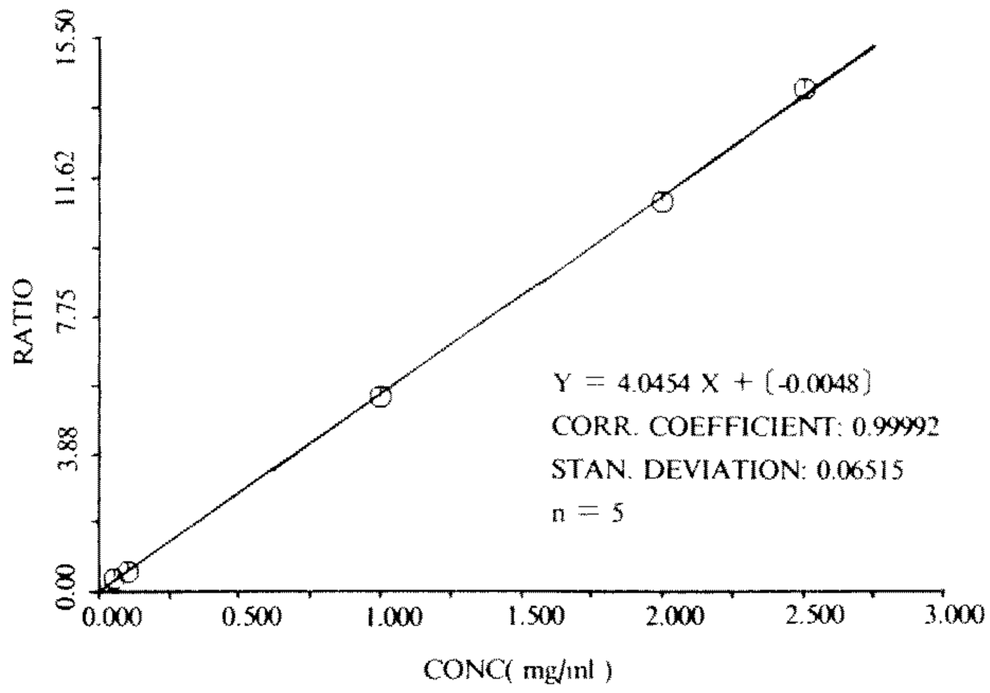

Figure 2. Standard curve for isosorbide 5-mononitrate. The points of curve are at 0.05, 0.1, 1.0, 2.0, and 2.5 mg/ml.

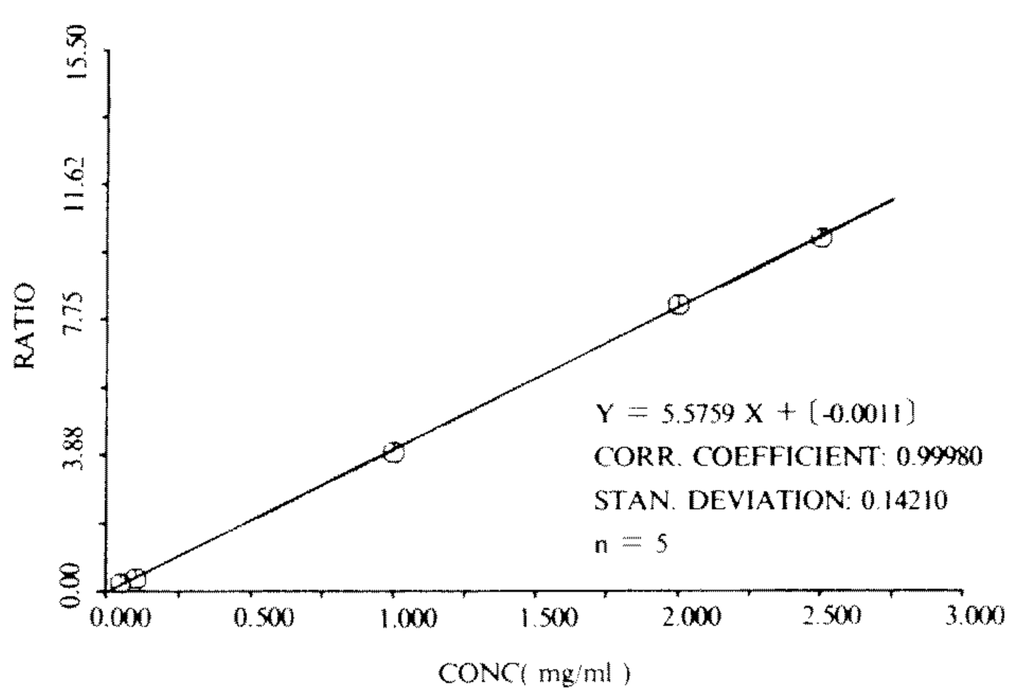

Figure 3. Standard curve for isosorbide dinitrate. The points of curve are at 0.05, 0.1, 1.0, 2.0, and 2.5 mg/ml.

not be detected. The results indicated that the proposed method is relatively unaffected by the sample matrix. The mean deviation of three nitrate esters, IS2MN, IS5MN and ISDN, was 0.01, 0.02 and 0.07, respectively. On the basis of recovery range, low RSD of reproducibilities and mean deviation demonstrate that the described HPLC method is good precision. Five samples of commercial formulations, including diluted bulk and various tablet dosage forms, were determined in the replicate for ISDN and IS5MN by the proposed HPLC procedure.

Preliminary chromatographic studies during the development of the proposed methodology explored the use of various non-buffered mixture composition (ie., 60% acetonitrile : 40% water) that was employed as same extract solvent. Analysis of standard solution under those conditions resulted in consistently low resolution of three nitrate esters. Therefore, a mobile phase composition of 0.005 M sodium hexanesulfonic acid, pH 4.75-acetonitrile (87:13, v/v%) was performed to analyze all tested solution. Typical chromatograms of the IS5MN and ISDN commercial dosage forms are shown in Figure 4. The retention time was 4.5 min for IS5MN, 5.8 min for 4-aminoacetophenone, and 28.5 min for ISDN. When compared with that obtained from standard mixture shown in Figure 5, no additional peak eluated as in the chromatogram. Table 2 was shown the results of varoius commercial pharmaceuticals the diluted bulk prepa-

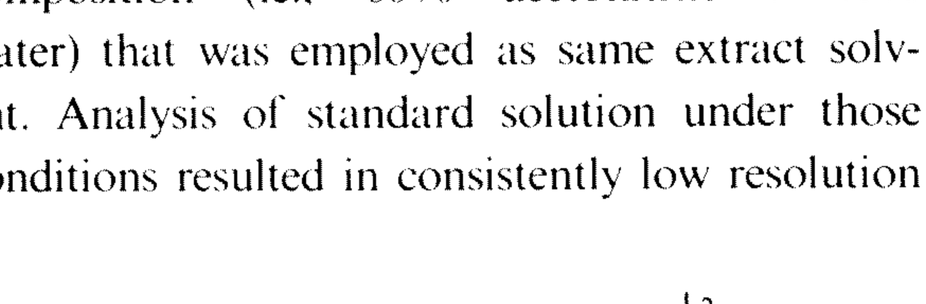

Figure 4. Chromatogram of isosorbide 5-mononitrate and isosorbide dinitrate preparations. (A)20 mg IS5MN tablet; (B) 5 mg ISDN Tablet; (c) 10 mg ISDN Tablet; (D) 20 mg ISDN retarded tablet; (E)working standard. Peak 1= IS5MN; 2=4-aminoacetophenone; 3 = ISDN; 4= IS2MN.

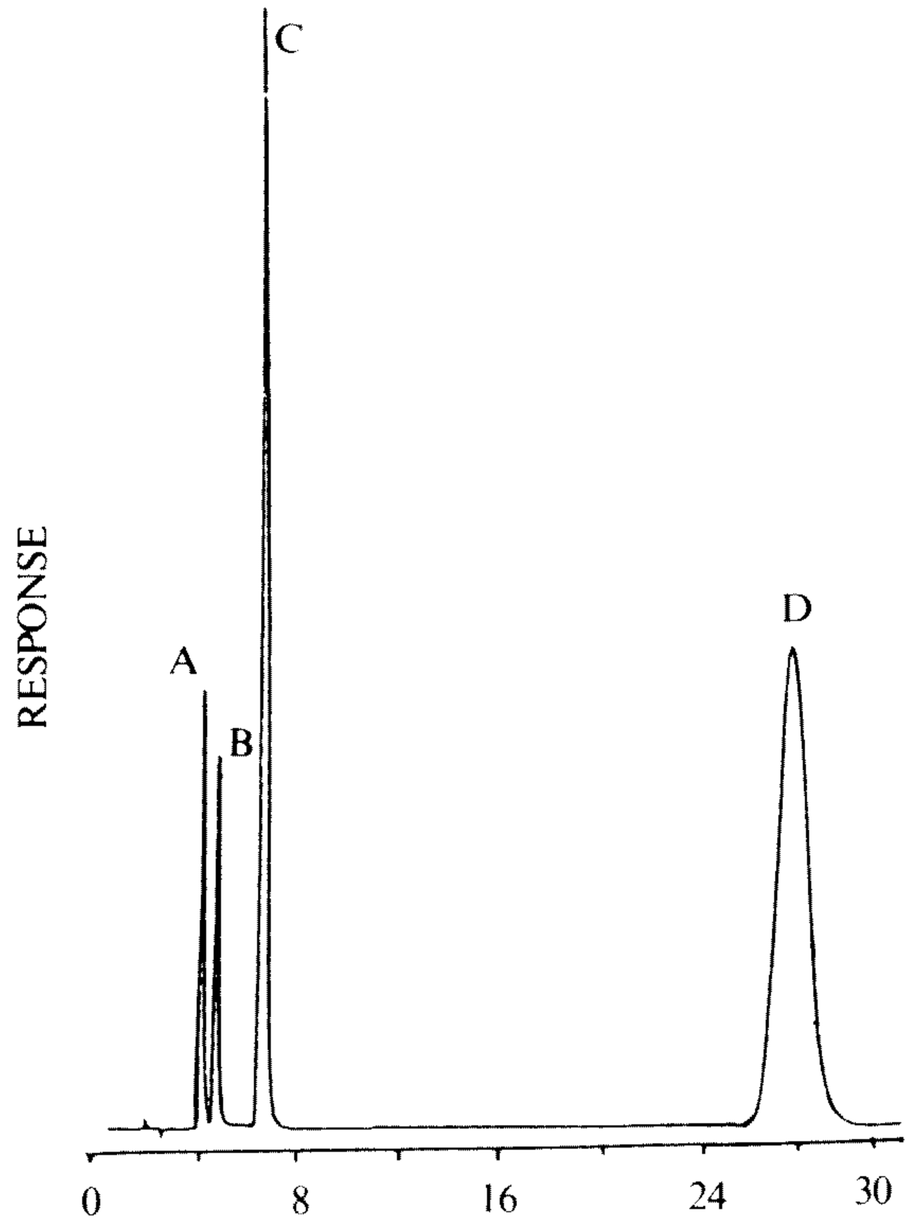

Figure 5. Chromatogram of a mixture preparation of IS2MN, IS5MN, ISDN and internal standard 4-aminoacetophenone under condition: Mobile phase: acetonitrile-20 mM PIC B6 (13:87) Column temperature was ambient; Peak identification A = IS2MN (2 ug), B = IS5MN (2 ug), C = Internal standard, 4-aminoacetophenone (2 ug), D = ISDN (2 ug).

Table 2. Recovery of IS5MN and ISDN from various commercial formulation Formulation

			mg labelled claimed	mg found	% (n=3) recovery	% average recovery
A	Tablet	IS5MN	20	19.94	99.7	99.5
				19.91	99.5	
				19.88	99.4	
B		ISDN	5	4.98	99.4	99.5
				4.97	99.4	
				4.98	99.6	
C		ISDN	10	9.96	99.6	99.4
				9.95	99.5	
				9.92	99.2	
D		ISDN *	20	19.90	99.5	99.6
				19.96	99.8	
				19.95	99.7	
E	Bulk	ISDN**	about 40	40.4	101.0	100.1
				40.2	100.0	
				39.81	99.5	

*ISDN as retard tablet

**Bulk ISDN was diluted with lactose and contained about 40% ISDN

ration. We found, including bulk drug, the recoveries were from 99.4 to 100.1% for triplicate injections. It was found to be fallen in the official requirement of 95% to 105% based on the labelled claim. Excipient from commercial formulations did not certainly interfere the results according to these recoveries.

It required extra time to agitate the tablet of retard formulation than usual oral tablet may that produce heating during shaken in the ultrasonic bath. The estimated sample preparation temperature and time for ISDN retarded tablet using shake in the ultrasonic water bath was determined to 44°C after three hours while normal tablet is accomplished in minutes. After sample of retarded tablet was heated, chromatogram of Figure 4-D was yield by performance the resulting mixture. As compared with the chromatogram, no additional peaks eluated at 3.8 min. for IS2MN that might be decomposed during heating as shown in Figure 5. The heating step required for one retarded product for matrix disposal did not affect the recovery of ISDN at all (i.e., no evidence of degradation) as illustrated in Table 1. The overall mean recovery from four different formulations was 99.6%.

Buffer concentration

The buffer concentration was found to be a key parameter for controlling the separation of isosorbide dinitrate and its analogues. Some observations indicate that HPLC method employing an aqueous-organic extraction solvent with similiar mobile phase without a buffering agent [17] may not be suitable for the assay of diluted bulk ISDN, nor for the finished dosage forms. It was not only possible to control the retention of isosorbide nitrates using buffer solution to

adjust eluent solvent strength, but also to shorten retained peak obtained. Plots of retention volume of ISDN, IS2MN, IS5MN and internal standard, 4-aminoacetophenone versus buffer concentration ranged from 0 to 40 mM were shown in Figure 6. Lower polarity of IS5MN and IS2MN they are, their almost flate plots were no remarkable variation at buffer concentration range from 0 to 40 mM, except at 5mM. To all analytes at buffer concentration from 0 mM to 40 mM, the retention volume were nearly no significant change. Higher polarity of ISDN and internal standard were dramatically fallen from buffer concentration between 5 and 20 mM, this illustrated that > 20 mM PIC B_6 not improved the retention. The peak shape of ISDN was broad while analyzed under condition containing without buffer reagent, but it improved increasingly with increase of buffer concentration. Although, without buffer addition, good separation and bad tailing of four compounds interested were observed. Expectedly, its retention was improved faster than that of no buffer and kept almost no change at buffer concentration larger than 20 mM. In contrast, the plots for the nitrate esters and internal standard were almost paralleled to the axis, except for ISDN.

pH Value

The pH dependence of separation was illustrated by the plot of retention volume versus pH at constant buffer concentration in Figure 7. Besides pH at 3.25, the pH effect of IS2MN was almost no change. For this effect of ISDN and IS5MN, the plots looked like "sigma" that they might be dissolved within pH 3.25 and 5.25. For pH 2.75, the longest retention for analyte was obviously longer than at 4.75. As has been observed with ISDN, increase in pH produced longer retention with ammonium acetate buffer[15]. Four analytes separated completely, however, it took longest retention at pH 3.25. Expectedly, pH had an effect on shortening ISDN retention from pH higher than 3.25 to 4.75. Mobile phase pH value within 3.25 and 5.25 was necessary for optimum resolution and peak shape. As has studied, slight decrease in pH produced longer retention and broader shape for ISDN than others. Better peak shape was

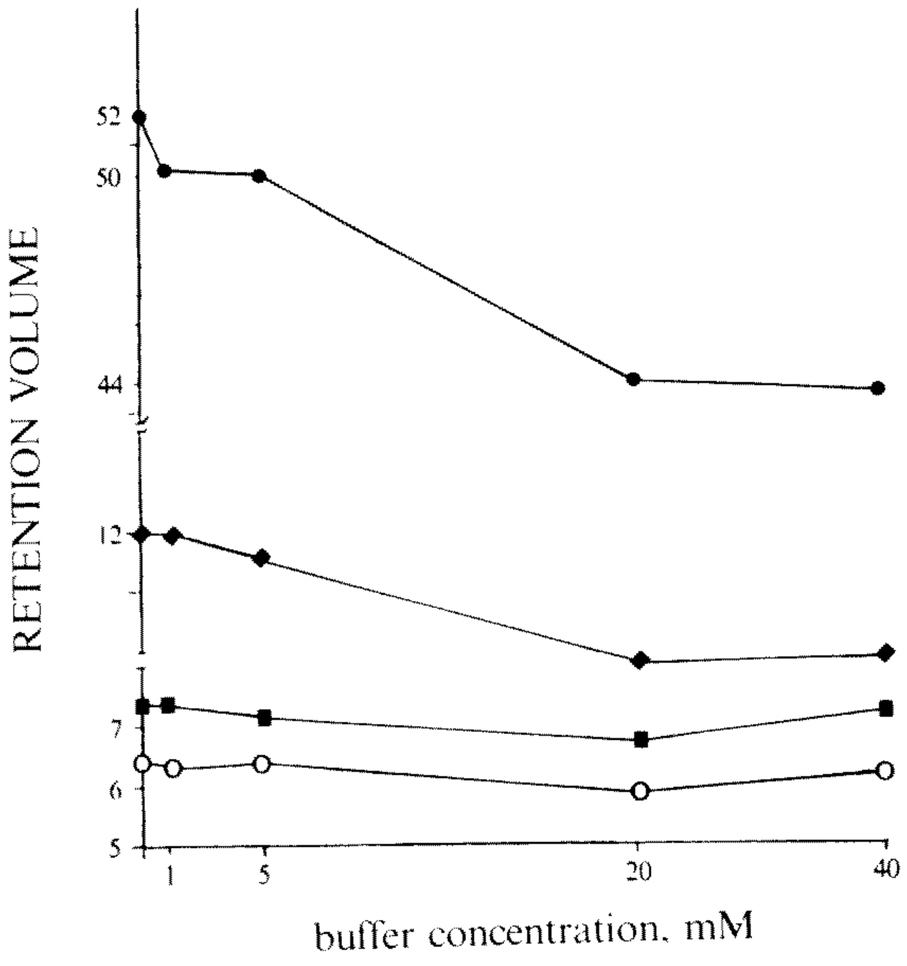

Figure 6. Effect of buffer concentration on retention volume of IS2MN, IS5MN, ISDN and 4-aminoacetophenone

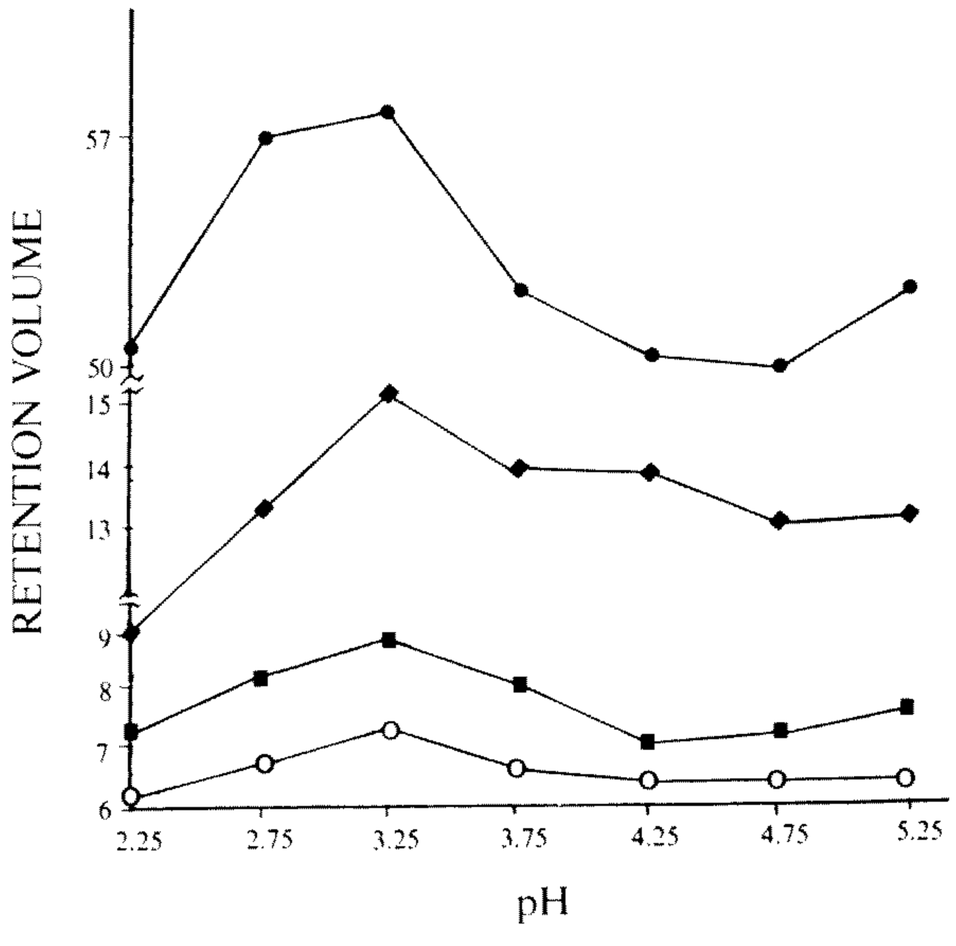

Figure 7. Effect of pH on retention volume of IS2 MN, IS5MN, ISDN and internal standard 4-aminoacetophenone.

also observed at lower pH value for nitrates. As expected, pH 2.25, four analytes were dissolved with a shortest retention. Another concern about the mobile phase pH should be careful to perform at very low pH, because too lower might damage the column at pH 2.25.

CONCLUSION

The conclusion is a reversed-phase column HPLC successfully separated Isosorbide dinitrate and its analogues. The separation was rapidly achieved within 30 min. by using a pH 4.75 PIC B6 buffer solution and acetonitrile on a Bondclone ODS column. The method was faster than a method B, described by M. Carlson[15], with a mobile phase composition of 0.2 M aqueous ammonium acetate-methanol-water (100:200:700,v/v/v%), pH 5.6, on 5 um column. Also, the current method separated four analytes interested better than that compendial method [16] was performed with mobile phase composition of 0.05 M ammonium acetate, pH 4.75-methanol-water(10:55:55, v/v/v%) as shown in Figure 8. It is also a specific and stability-indicating in regard to the IS5MN and IS2MN degradation product. It can be applied to most currently marketed pharmaceutical products containing ISDN and IS5MN at minimum concentration of 0.05mg/ml (0.05 ug). The PIC B_6 buffer concentration and the pH could mainly control the retention time and the peak shape of ISDN. However, there are less buffer effects on the other nitrate esteres study.

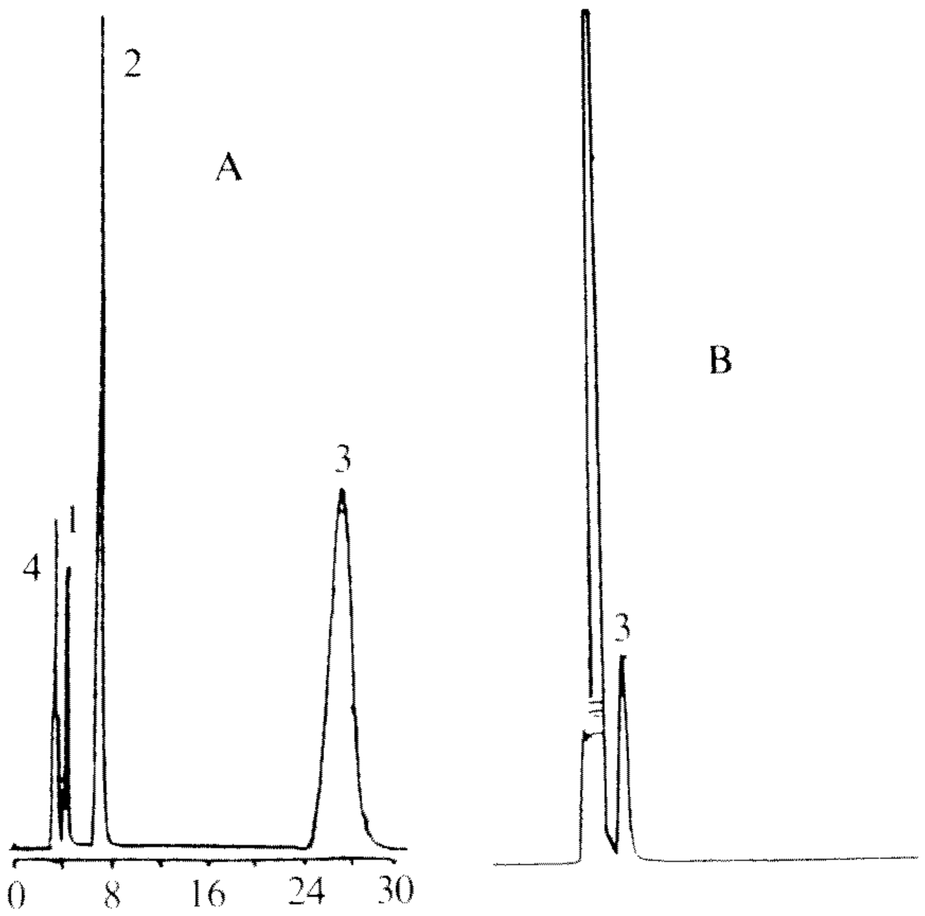

Figure 8. Chromatogram of a mixture of isosorbide 5-mononitrate, isosorbide 2-mononitrate, isosorbide dinirtate and internal standard 4-aminoacetophenone under condition: (A)mobile phase of 20 mM PIC B6 (pH 4.75)-acetonitrile (B)mobile phase of 0.05M ammonium acetate solution(pH 4.75)-methanol-water (10:55:55)

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應用液相層析法測定Isosorbide Nitrate及其類似物與其製劑

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摘　要

逆相高效液相層析法已發展利用於鑑別及測定 isosorbide dinitrate (ISDN), isosorbide 5-monoitrate (IS5MN) 及其降解物isosorbide 2-mononitrate (IS2MN),同時應用於分析此二成分之製劑。

整個高效液相層析系統由Waters 600型幫浦,700型之自動檢體採樣機與高容量檢品注射圈活瓣、486型可變波長之紫外光偵測器,偵測波長設定於220 nm及用於圖譜積分之745 B型積分儀組成。以C18 (3.9 mm X 250 mm, 10 um i.d.)之層析管於同濃度移動相pH 4.75含sodium hexanesulfonic acid-acetonitrile,流速1.5 ml/min.於三十分鐘內能將ISDN, IS5MN,內部標準品4-aminoacetophenone及ISDN之次要代謝物,亦爲降解物完全分離並予以定量,本研究所用之方法僅須單一移動相即能將所欲分離isosorbide nitrate esters完全分離。

0.05至2.5 mg/ml之標準溶液稀釋液之校正係數分別測定如下:ISDN, r = 0.9998及標準偏差0.48 %; IS5MN, r = 0.9999及標準偏差0.08%; IS2MN, r = 0.9999及標準偏差0.05%。由市售口服錠、持續錠及乳糖稀釋之ISDN原料藥應用本研究所發展之方法其回收率均大於98%。因此研究所使用之分析方法快速、簡單又確實可用。