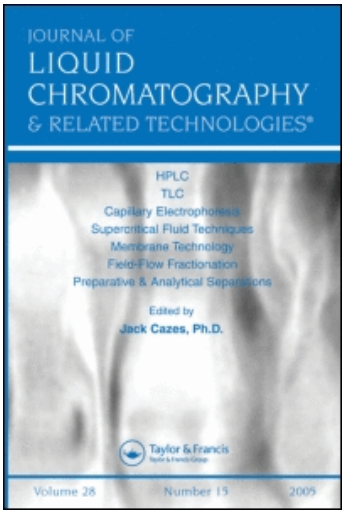

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Agbaba, Danica , Janjic, Valentina , Zivanov-Stakic, Dobrila and Vladimirov, Sote(1994) 'High Performance Liquid Chromatographic Assay for Isosorbide 5-Mononitrate and Impurities of Inorganic Nitrates in Pharmaceuticals', Journal of Liquid Chromatography & Related Technologies, 17: 18, 3983 — 3988
To link to this Article: DOI: 10.1080/10826079408016167
URL: http://dx.doi.org/10.1080/10826079408016167

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(18), 3983-3988 (1994)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR ISOSORBIDE 5-MONONITRATE AND IMPURITIES OF INORGANIC NITRATES IN PHARMACEUTICALS

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ABSTRACT

A simple and reliable high performance liquid chromatographic (HPLC) method for the quantification of isosorbide 5- mononitrate and inorganic nitrate impurity in raw material and dosage formulations has been developed and validated. The procedure which simultaneously resolves active organic nitrates : isosorbide 5-mononitrate, isosorbide 2-mononitrate and isosorbide 2.5-dinitrate , and main impurities (including related nitrate and acetate esters) , may be used as a control of purity of raw material and dosage forms.

INTRODUCTION

Coronary vasodilator isosorbide dinitrate (ISDN) was found to metabolize the following active mononitrates : isosorbide 5-mononitrate (5-ISMN) and isosorbide 2-mononitrate (2-ISMN). The former one has been recently found to be more active or less toxic and therefore predominantely used in the treatment of angina pectoris.

Stability studies (1-4), identification or determination (5-24) of mono- and dinitrate esters in raw material, as well as in dosage formulations, have been mostly performed by using HPLC (1,3,5-10,24), GC (2,4,11-12) or TLC (13-17). Colorimetry (18-19) and polarography (20-22) have also been used for quantification of organic nitrates in dosage forms. Isosorbide 2-acetate (IS-2A), 5-acetate (IS-5A) and diacetate (ISDA), 2-nitrate-5-acetate (IS-2N5A) or 2-acetate-5-nitrate (IS-2A5N) could be traced as impurities originated from the synthesis of parent nitrates. The above mentioned mixture was isolated and identified by GC(23).

According to British Pharmacopoeia (18) it is required to analyse the presence of inorganic nitrates in raw material and dosage forms.Inorganic nitrates can derive as impurities either from the synthesis of organic nitrates or as a product of degradation.

However, there are no published reports on the quantification assay of inorganic nitrates impurities. Therefore, the present paper will focus on assay of 5-ISMN and the isolation and quantification of the residue of inorganic nitrates in raw material and dosage forms by HPLC.

EXPERIMENTAL

Reagents

Isosorbide 5-mononitrate (5-ISMN), isosorbide 2-mononitrate (2-ISMN) and isosorbide 2.5-dinitrate (ISDN) , isosorbide (IS), isosorbide 2-acetate (IS-2A), isosorbide 2.5-diacetate (IS-2.5DA), isosorbide 2-acetate-5-nitrate (IS-2A5N) were obtained from Kali Chemie Pharma (Hanover,FRG). Sodium nitrate was of analytical grade. Water and methanol were of HPLC grade. Ancorbid^{R} sustained release pellets, containing 40 mg and 60 mg 5-ISMN, were obtained from "Zdravlje" Leskovac (Serbia) .

Instruments

HPLC system Spectra Physics (San Jose ,CA,USA) Model 8100 was used equipped with a UV detector Model 8440 (wavelength set up at 220 nm) and an integrator Model 4200. The analytical column (250 x 4 mm) was packed with 5 μm Lichrosorb RP 18 (Merck, Darmstadt,FRG). The mobile phase of methanol-water (30 : 70) was filtered through a 0.45 μm membrane filter and degassed in an ultrasonic bath prior to use. The flow rate was 1 ml/min.

5-ISMN and Inorganic nitrates impurity assays.

A stock solution of 1 mg/ml 5-ISMN was prepared in water; calibration solutions were prepared by diluting the stock solution to obtain 0.1-0.25 mg/ml. Working standard solution of 5-ISMN was diluted to the concentration of 0.2 mg/ml.

A stock solution of 0.5 mg/ml sodium nitrate was prepared in water; calibration solutions were prepared by diluting the stock solution to obtain 0.25 - 2.5 μg/ml.

Sample solutions - The amounts of raw material or powdered pellets containing 50 mg of 5-ISMN were dissolved in 100 ml water. After filtration, the aliquots of 50 μl sample solution were subjected to HPLC for an inorganic nitrates assay. 50 μl of the diluted sample solution to 0.2 mg/ml were subjected to HPLC for 5-ISMN assay.

RESULTS AND DISCUSSION

Separation of 5-ISMN from active nitrates 2-ISMN, ISDN, and potential impurities, arose from different sources such as :IS (starting material in the synthesis), IS-2A, ISDA, IS-2A5N (intermediates in the synthesis), and inorganic nitrates (degradation products), is shown in Fig.1. It can be observed that under the conditions of the procedure, IS and inorganic nitrates have similar retention time (about 1.6 and 1.7 min., respectively). However, inorganic nitrate has a lower detection limit (factor 10^3) than IS and its determination from diluted samples cannot be interfered by IS.

Validation of the method

Linearity

The response (peak area) was proportional to the concentrations over the range tested; between 0.1 and 0.25 mg/ml for 5-ISMN and 0.1 to 5 μg /ml for inorganic nitrates (equivalent to 0.1 -2 % of the 5-ISMN concentration). The regression equations were $y= 4.3 +1944x$ with a correlation coefficient of $r=0.999$ ($n=5$), where x=mg/ml and $y= 4 + 224x$, and $r=0.998(n=5)$, where x=μg/ml for 5-ISMN and inorganic nitrates,respectively.

Precision

The repeatability of the analytical system was determined by using two samples of 5-ISMN found to contain 0.176 and 0.5% of inorganic nitrates impurities. Six consecutive replicate injections of each sample gave a relative standard deviation (RSD) of 2.7 and 0.9%. The repeatability of 5-ISMN assay running the concentrations of 0.1, and 0.2 mg/ml, gave a RSD of 1.5 and 0.7%.

Recovery

The accuracy of the method was proved by determination of 5-ISMN from laboratory-made tablet excipient mixture spiked with a 5.3, 14.8 and 24.4 mg of 5-ISMN: recoveries were 100.26, 100.67 and 100.09, respectively. A solution containing 5-ISMN with no detectable amount of inorganic nitrates was spiked with aliquots of the impurity solution

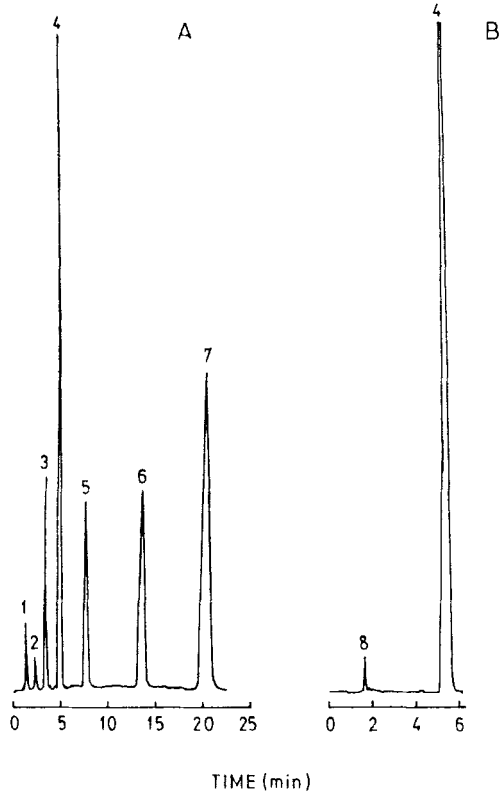

FIGURE 1. HPLC chromatogram of: A - mixture of IS (1), 2-ISMN (2), IS-2A (3), 5-ISMN (4), IS-2.5DA (5), IS-2A5N (6) and ISDN (7), B - sample of Ascorbid 40 mg pellets containing inorganic nitrates (8) at a concentration of 0.18%. The retention times were 1.60, 2.20, 3.42, 5.03, 7.95, 14.30, 21.50 and 1.70 for compounds 1,2,3,4,5,6,7 and 8 respectively,

TABLE 1.

Assay of 5-ISMN and Inorganic Nitrates

	5-ISMN		Inorganic Nitrates	
	Taken (mg)	Found mg ± s. d.	%	RSD[a]
Raw Material	50	49.2 0.75	0.11	4.5
Ascorbid 40 mg tbl.	40	38.9 0.75	0.17	4.7
Ascorbid 60 mg tbl.	60	58.9 0.97	0.20	3.5

[a]Relative Standard Deviation

at two concentrations of 1.25 and 5 μg/ml (equivalent to 0.5 and 2%). Recoveries obtained were 102.5% and 100.5%. Detection limit of the method was 1.5 μg/ml for 5-ISMN and 0.065 μg/ml (equivalent to 0.024%) for inorganic nitrates.

Application

The method was used to screen the raw material and dosage forms on inorganic nitrates impurities. The results obtained for 5-ISMN and inorganic nitrates assay are shown in Table 1. The results obtained for inorganic nitrates of 0.11% for raw material and 0.17 - 0.2% for dosage forms meet the requirements of USP not exceeding 2%.

The results suggest that because of its sensitivity and reproducibility, the method may be suitable for the simultaneous determination of 5-ISMN and inorganic nitrates impurities in raw material and dosage formulations.

Simple isocratic system used for separation is found to be suitable for routine purity control of active nitrates.

ACKNOWLEDGEMENT

The authors wish to thank Miroslav Nikolic and Verica Nikolic for the kind contribution in experimental part of the work.

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Received: February 18, 1994
Accepted: May 24, 1994