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Short communication

Direct injection of aqueous samples in packed column supercritical fluid chromatography of isosorbide-5-mononitrate from drug release testing☆

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Abstract

A method for the analysis of aqueous samples of isosorbide-5-mononitrate (5-ISMN) is presented. It is based on packed column supercritical fluid chromatography (SFC) using 20% of 2-propanol in carbon dioxide as the mobile phase and a diol silica column as the stationary phase. Using the described conditions it is possible to quantitate 5-ISMN released from Imdur® tablets in gastric media. The precision upon repeated injections was 2% (RSD) at the 20 μg/ml level ($n = 8$), using peak height measurements, when the solution was circulated through the sample loop of the injector. Samples from drug release testing that had been analyzed with reversed phase LC were analyzed with the present method and the results agreed well. It is also possible to monitor the drug released in a dissolution-testing vessel through direct on-line continuous loading (recirculation) of the sample loop of the SFC instrument.

Keywords: Packed column supercritical fluid chromatography; Aqueous injections; Isosorbide-5-mononitrate; Drug release analysis

1. Introduction

Packed column supercritical fluid chromatography (SFC) can be used for the determination of isosorbide-5-mononitrate (5-ISMN) and related compounds in the bulk drug substance and in tablets. Methanol was used as polar organic modifier and porous graphitic carbon as column support (Hypercarb®) [1]. Tetramethyl-ammonium hydrogen sulphate was included in the modifier in order to elute inorganic nitrate. The sample preparation was simple; i.e. the bulk substance, or ground tablet powder, was dissolved in methanol prior to injection.

Since SFC with packed columns generally shows a normal phase-like chromatographic behaviour aqueous samples are regarded with suspicion. This is because most normal phase systems are sensitive towards water. They need long equilibration times and/or the system can be temporarily affected by water present in the sam-

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ple medium. Also in packed column SFC aqueous samples are generally avoided. We have circumvented the problem of aqueous injections in SFC by replacing the by far most commonly used modifier methanol with 2-propanol [2]. The 2-propanol–carbon dioxide mobile phase mixture seems to accommodate adequate water sample volumes and the column efficiency is not reduced [2]. On the other hand, under certain conditions peak compression [3,4] can be generated and excellent apparent plate numbers obtained [5,6]. Using a semi preparative 250 × 10-mm ID Hypersil column 150 μl of 80% water in 2-propanol could be loaded into the SFC system with clevidipine as analyte [7]. Some alcohol was needed in the sample solution in order to keep the analyte in solution.

Packed column SFC is perhaps not a first hand choice for new analytical methods for samples dissolved in water, but the strength in having duplicate analytical tools based on different separation principles is a sound advantage in the development of methods for new drugs. The purpose of this short communication is to show the possibility to use packed column SFC for the determination of 5-ISMN released from Imdur® tablets in gastric media including the option to continuously sample and analyze the drug from dissolution testing baths. The results show that it is possible to use a normal phase chromatographic technique for direct dissolution testing of 5-ISMN tablets.

2. Experimental

2.1. Supercritical fluid chromatograph

The instrument used for SFC was from Hewlett-Packard (Little Falls Site, Wilmington, DE, USA). It had been upgraded with a BI3100 liquid sampler handler (Berger Instruments, Newark, DE, USA), which corresponds to an Alcott Model 718 sampler. The software was BI-SFC Chemstation version 3.3.6 for Windows 95. The Valco injector was equipped with an external 5 μl sample loop. The UV-signal at 214 nm was measured throughout in this study.

Pumps for passing the sample solution through the external loop of the injector were as follows: an ISCO μLC-500 syringe pump with 50 ml capacity (Lincoln, NA, USA) and an LKB 2150 pump (Bromma, Sweden). The latter was used to continuously circulate sample liquid from dissolution baths. During early development work a peristaltic pump for plastic tubing was also used; Ole Dich Pump 103 (Hvidovre, Denmark).

2.2. Columns, reagents and chemicals

The chromatographic columns were standard LC ones as follows: Hypercarb® 100 × 4.6 mm ID (Hypersil, Astnoor, UK), LiChrosorb RP-8 125 × 4 mm ID (E. Merck, Darmstadt, Germany), LiChrosorb RP-18 125 × 4 mm ID, LiChrospher 100 diol 125 × 4 mm ID (E. Merck), Kromasil 150 × 4.6 mm ID (EKA-Nobel, Bohus, Sweden). The particle size was 5 μm. Solvents used were of LC-grade quality. The carbon dioxide was 2.7 grade with a dipper tube in the cylinder from AGA (Lidingö, Sweden). USP gastric media without enzymes was prepared by mixing 175 ml of concentrated hydrochloric acid and 50 g of sodium chloride with 25 l of water, the pH was ∼1.2.

Imdur® 120 mg tablets, reference bulk substance and related substances (isosorbide-2-mononitrate and isosorbide-2,5-dinitrate) were obtained from AstraZeneca Operations (Södertälje, Sweden).

3. Methods

The preliminary screening for a suitable column utilised an oven temperature of 40 °C, a flow-rate of 1.5 ml/min of 10% 2-propanol in the carbon dioxide and a back pressure of 150 bar. The final method was 1.0 ml/min of 20% 2-propanol at 40 °C with the backpressure set to 100 bar.

The dissolution vessel with 500.0 ml of gastric media was an USP apparatus type 2. For the present studies no thermostating was used and a magnetic bar, 40 × 8 mm large, was utilised for agitation of the media, instead of the commonly used rotating paddles.

For continuous loading of the sample loop the moving needle of the BI3100 injector was removed, and the needle guide of the injection port of the valve was replaced with stainless steel tubing from the ISCO or LKB pumps. These pumps were run continuously and no precaution was taken in the sample line upstream the valve to prevent backflush of carbon dioxide gas bubbles into the sample solution.

4. Results and discussion

4.1. Screening for a suitable column

As discussed in the introduction replacing methanol with 2-propanol will facilitate the analysis of aqueous samples. However, the chromatographic separation of 5-ISMN on Hypercarb® resulted in peaks with marked tailing even with as high 2-propanol modifier content as 20%. The symmetry of the 5-ISMN peak did not improve at elevated temperature either. Up to 80 °C was investigated. The precision upon repeated injection of an aqueous solution at 30 μg/ml was poor with a RSD of 6.7% for five injections. Both Kromasil bare silica and LiChrosorb RP-8 gave good peak symmetry and good column efficiency but system peaks generated by water interfered with the organic nitrate ester peak and could not be sufficiently eliminated, or taken advantage of. Since quantitation appeared to be more of a problem at lower concentrations some attempts to use a system based on RP-8 as support were performed but even at higher levels of 5-ISMN the precision was poor. Using LiChrosorb RP-18 the system peaks from water injections were even more pronounced and extended in time during the chromatographic run.

4.2. Chromatographic system using diol silica

Symmetric peaks with good efficiency were obtained on a diol column for 5-ISMN injected dissolved in 2-propanol (Fig. 1). Using water, or gastric media, the rather pronounced system peak was well separated from the 5-ISMN peak though in the beginning the return to baseline was rather slow, about 3–4 min, and not as distinct as in Fig. 1. Comparison of standards prepared with 2-

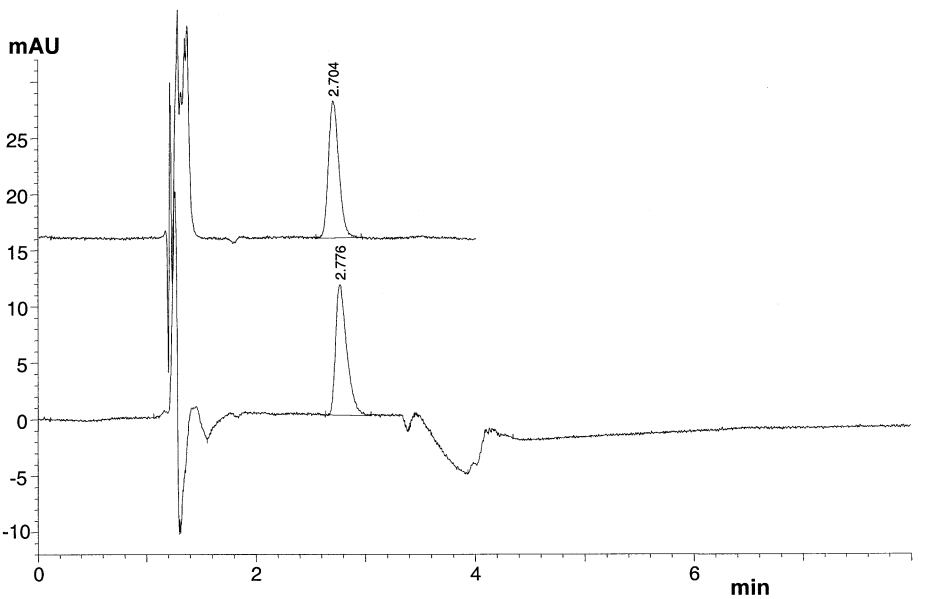

Fig. 1. SFC after injection of 5-ISMN dissolved in 2-propanol 30 μg/ml (upper trace) and gastric media (lower trace). Chromatographic conditions: see the Section 2.

Table 1
Precision data from continuous on-line loading of sample solutions of 5-ISMN from a dissolved Imdur 120-mg tablet

Concentration (μg/ml)	RSD% ($n = 8$)	
	Area	Height
63	3.96	0.84
36	3.97	1.83
21	3.82	1.95

Chromatographic conditions in the Section 2, diol column. Sample fed using an ISCO μLC-500 pump at 100 μl/min flow rate.

propanol and gastric media, respectively, revealed no difference in the measured area but consistently between 2 and 3% longer retention times for the aqueous standards. One explanation could be that the injected water expels some adsorbed 2-propanol from the pseudostationary phase, and that free diol-groups retain the analyte more as compared with when organic solvent samples are injected and no free hydroxyls are generated. Furthermore, the peak width was some 6% larger for nine injections at the 30 μg/ml level as compared with the same concentration dissolved in 2-propanol. This is not surprising since it is common to avoid too solvating solvents for the sample if possible, in order to prevent unnecessary band broadening.

4.3. On-line sample loading

The possibility to use the method for the analysis of 5-ISMN in gastric media was evaluated using a syringe pump that was loaded with a solution taken at three early intervals from a stand alone dissolution testing of an Imdur® tablet. The precision data are presented in Table 1 and show that adequate relative standard deviations were obtained down to 21 μg/ml with the present method, if the evaluation was done with peak height measurements and not by area. Since the RSD% was rather high even at 63 μg/ml it is believed that the integration of the area is not the cause but the presence of water in the sample at the moment of injection into the system.

Previous work using on-line loading, of a viscous aqueous sample, necessitated an on/off valve inserted in the sample line in order to prevent bubbles of carbon dioxide to expand upstream [8]. That is the valve was closed some time before the valve was returned to the load position and released gaseous carbon dioxide could thus escape through the waste port only. That problem is more likely to be present with the low flow-rates used earlier [8].

4.4. Drug release

The method was applied to samples that had been taken from a regular in-house dissolution-test experiment. These samples had been analysed by reversed phase liquid chromatography [9]. The average of these six samples, from each time of withdrawal, is given in the graph in Fig. 2 together with the corresponding average from the LC method. From the Fig. 2 it is evident that the agreement is good and that this packed column SFC method could have been an alternative to LC if it had been available at the early time of drug development when such decisions are taken. One can also conclude that the yield of this SFC method is not different compared with that of the LC one.

The method was also tested during an experiment where the medium was circulated continuously through the injection valve of the SFC instrument. Fig. 3 shows both the blank chromatograms, before the tablet was added, and the first chromatogram recorded after 20 min. The 5-ISMN peak corresponds to about 15 μg/ml. The water generated system peak is less than 2 min wide (Fig. 3). No extraneous peaks were observed from 5 min and longer which means that a new injection is possible every 5 min. Fig. 4 shows the data points obtained from the sampling close to 1, 2, 4 and 6 h. The full curve with all data points showed a slight winding around the curve which can be attributed to uneven temperature or mixing in the USP-bath by the magnetic bar, or unevenness of the tablet, which becomes visible with short sampling intervals, 14 min.

The selectivity of the present method was proven with the related substances isosorbide-2-

mononitrate and isosorbide-2,5-dinitrate. Their selectivity factors were found to be 0.4 and 0.5, respectively. This means that they elute earlier, and that they will not interfere with the peak from 5-ISMN. Nitrate is a possible degradation product but does not elute within k 30. The signal

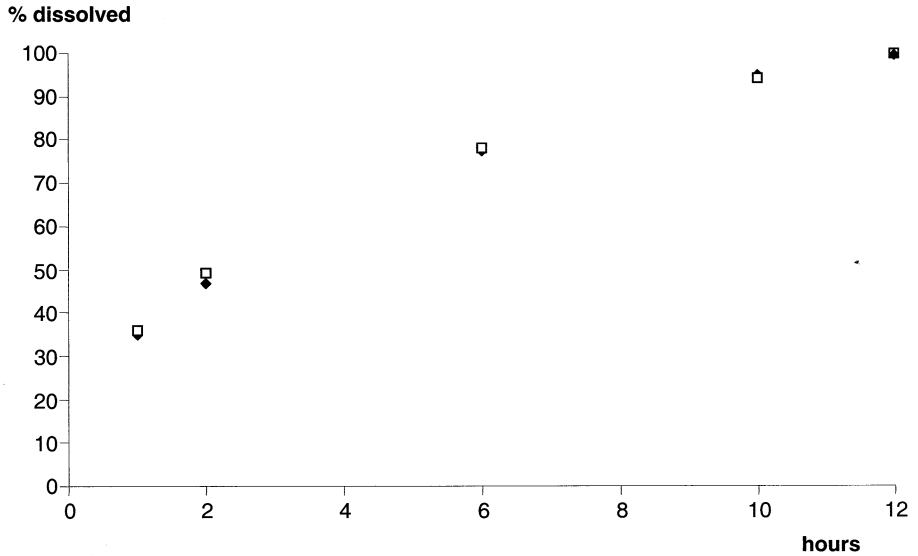

Fig. 2. Release of 5-ISMN from an Imdur® 120 mg tablet. Samples were withdrawn and analysed by SFC (◆) and LC (□). Each point is the average of single chromatographic determinations from six vessels containing one tablet each per 500.0 ml of gastric media.

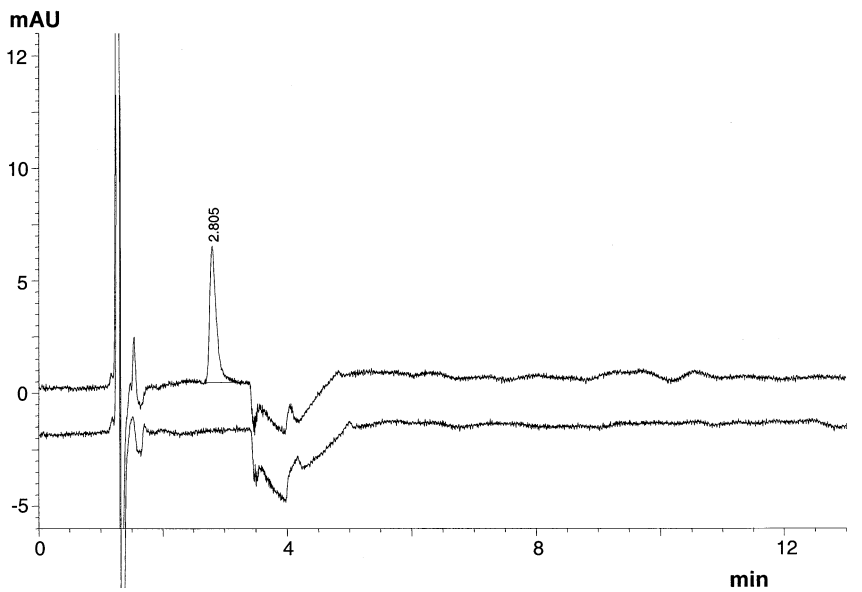

Fig. 3. Chromatograms from direct continuous on-line loading/injection from a dissolution-testing bath. Lower SFC trace blank media before the tablet was added to the bath. Upper SFC traces 20 min after an Imdur® 120-mg tablet had been added. Recirculation using an LKB-pump at 0.3 ml/min. chromatographic conditions: see the Section 2.

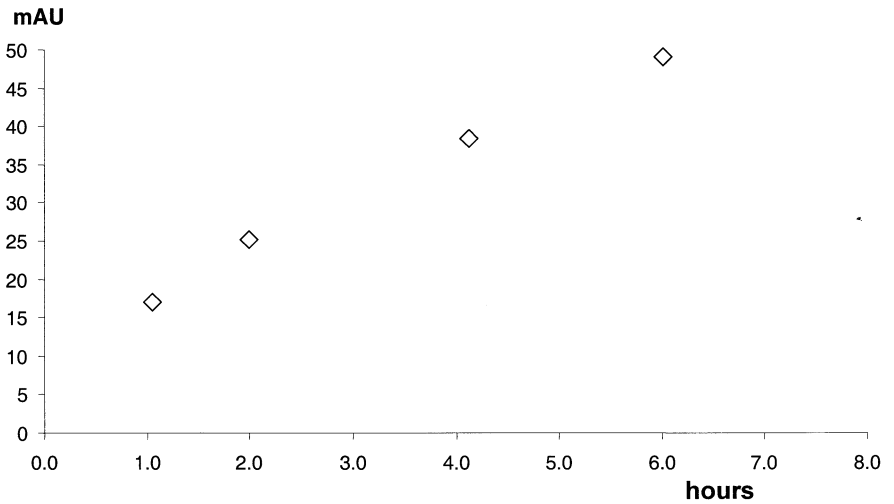

Fig. 4. Release of 5-ISMN from an Imdur® 120-mg tablet from direct continuous on-line loading/injection from a dissolution testing bath (cf Fig. 3 above).

to noice ratio (S/N) at 21 μg/ml level mentioned above was about 28. One can thus assume that the present method will be adequate also for 30 mg tablets at the 1 h sampling time, especially since drug release is measured at several time intervals.

5. Conclusions

This short communication shows that it is possible to analyse aqueous samples containing 5-ISMN both from samples withdrawn during drug dissolution testing and also from sampling through continuous loading, and recirculation, of media from such a bath. These results demonstrate that packed column SFC is a viable separation technique that can be used as an alternative, or complement, to regular reversed phase liquid chromatography when a normal phase like system is required. The different selectivity of this system is evident from the fact that the dinitrate ester elutes twice as fast whereas for the RP LC method almost 30 min is required.

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