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Packed-column supercritical fluid chromatography of omeprazole and related compounds

Selection of column support with triethylamine- and methanol-modified carbon dioxide as the mobile phase☆

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ABSTRACT

Using standardized chromatographic conditions with respect to carbon dioxide and methanol containing 1% of triethylamine as modifier, the chromatographic behaviour of omeprazole and four of its analogues was investigated on six types of chromatographic support at 40°C. Superior selectivity was obtained for omeprazole *versus* its analogues with NH_2-modified silica and also good peak symmetry (LiChrosorb and Kromasil). Using the present chromatographic system it is possible to detect and separate the analogues added to omeprazole in the 0.5% (w/w) range within 10 min.

INTRODUCTION

Omeprazole (Fig. 1) is a gastric proton-pump inhibitor (Losec). The methods used for the control of the stability of the substance and its content in the pharmaceutical formulations are based on normal-phase liquid chromatography with UV detection using a silica column and dichloromethane containing methanol and ammonia as the mobile phase [1,2]. For omeprazole sodium a reversed-phase system with acetonitrile–water as the mobile phase and Microspher C_{18} as the support is preferred [3]. We were interested in exploring whether it was possible to use modified carbon dioxide as the mobile phase for the chromatography of omeprazole and related compounds. As capillary columns have limited sample capacity and the compounds of interest here are polar and thermolabile we preferred packed columns.

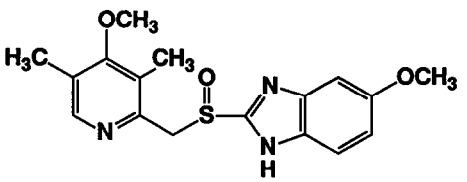

Fig. 1. Structure of omeprazole. The methoxy group of the pyridine can be absent (as in H 180/29) and the nitrogens of the imidazole can be methylated (isomers, H 193/61).

Supercritical fluid chromatography (SFC) has attracted considerable interest for the separation and determination of a wide range of different compounds [4]. The greatest number of publications have been based on studies using capillary columns

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and the analytes are often fairly lipophilic in nature. Another reason is that most commercial instruments have been constructed with capillary columns or narrow-bore packed columns.

For improved sample capacity and speed of analysis, packed columns are attractive. Howevere, pure carbon dioxide and ordinary columns packed with silica particles are not useful for most polar and protolytic compounds. Therefore, polar modifiers are mixed with the mobile phase. They work both by deactivating active sites on the support, mainly acidic silanol groups, and by increasing the solvating power of the mobile phase [5–8]. The most frequently used modifier is methanol. As our compounds of interest have basic nitrogens we included a tertiary amine in the main modifier, as is common practice in liquid chromatography [9].

Most of the supports used for packed-column SFC were originally intended for liquid chromatography. Often a certain type of column is chosen without any experimental evidence for its superiority, although some exceptions where different supports have been investigated in detail have been published [10–17]. For pharmaceutical substances the most commonly used supports seems to be the cyano [16,18–22] and amino [10,22–27] types of modified silicas. The silica can also be modified for SFC use but from the reported chromatograms it seems clear that residual activity from the support still persists [28,29]. Papers have also been published on the use of polymeric supports with SFC [11,20,30,31] but their retaining properties are high and they suffer from being sensitive to repeated pressure changes [31].

The aim of this work was to evaluate and select a suitable chromatographic support for the separation and determination of omeprazole and some of its possible contaminants and degradation products.

EXPERIMENTAL

Instrumentation

The chromatographic system was constructed from the following separate units. The pump for CO_2 was an LKB (Bromma, Sweden) Model 2150 with pump heads cooled by a circulating water–glycol mixture (2°C) (MGW Lauda RM6, Lauda-Königshofen, Germany). A jacket in the form of an E was shaped from a block of aluminium to fit snugly on the pump heads and made hollow by drilling several holes at different angles and plugging all except two (inlet and outlet) with screws. A second pump, ISCO (Lincoln, NE, USA) Model μLC-500 was used for delivery of the modifier. Carbon dioxide and the modifier were merged in a tee using tubing that almost faced each other. Mixing was accomplished using a magnetic stirrer in a small vessel (*ca.* 1.5 ml), previously used as slurry reserve.

A Rheodyne (Cotati, CA, USA) Model 7010 injector with a 20-μl loop was used for sample introduction. The columns were kept in a water-bath at 40°C (Thermomix 1441; Braun, Melsungen, Germany). The detector was a Jasco (Tokyo, Japan) UV 875 with a high-pressure cell (4 μl). The wavelength monitored was 300 nm and the absorbance range 0.04. A third pump was connected to the system downstream of the detector as a pressure monitor (ISCO μLC-500). A piece of fused-silica tubing (15–20 cm × 50 μm I.D.) was used as restrictor. To prevent clogging by solid carbon dioxide, most of it was kept in the water-bath. A Hewlett-Packard Model 3390A or a Spectra-Physics Model 4400 integrator was used to display the signal from the UV detector.

Columns

Standard commercial liquid chromatographic columns were used. Initially they were tested with pure carbon dioxide and polyaromatic hydrocarbons (naphthalene, anthracene and pyrene) as test substances dissolved in hexane prior to chromatography with modifiers in order to check the efficiency (wavelength 254 nm, 0.04 absorbance). The following dimensions and sources were used: 125 × 4.0 mm I.D. (NH_2, DIOL, CN, RP-18, Si-60; Merck, Darmstadt, Germany); 125 × 4.6 mm I.D. (NH_2; Eka Nobel, Bohus, Sweden); 200 × 4.6 mm I.D. [NH_2 and $N(CH_3)_2$; Macherey–Nagel, Düren, Germany]; and 150 × 4.6 mm I.D. (CN; Supelco, Bellefonte, PA, USA). The size of the particles was 5 μm throughout.

Chemicals and reagents

The carbon dioxide used was 3.5 grade with dipper tube from AGA (Lidingö, Sweden), methanol and dichloromethane of analytical-reagent grade were obtained from Merck and triethylamine zur Synthese from Merck was glass distilled.

Omeprazole, omeprazole sodium and the corresponding sulphide (H 168/22), sulphone (H 168/66), N-methylated (H 193/61) and desmethoxylated (H 180/29) (the pyridine) compounds were from the Department of Organic Chemistry, Astra Hässle (Mölndal, Sweden). Their structures can be understood from that of omeprazole in Fig. 1.

Methods

The substituted benzimidazoles were dissolved in dichloromethane at *ca.* 500 μg/ml. Samples were chromatographed using a pump flow setting of CO_2 of 1.20 ml/min and modifier of 60 μl/min (1% of triethylamine in methanol). The system was allowed to equilibrate for about 1 h before any measurements were made. For each column the separation factors with respect to omeprazole were calculated from the capacity factors, $k' = (t_r - t_0)/t_0$. When possible this was done from the injection of a complete mixture of the compounds of interest. The hold-up time was measured from the point of injection to the apex of the negative peak caused by the injection. The symmetry was calculated up one tenth of the peak height from the baseline. Normally a minimum of two new columns of each type were investigated in order to confirm the results obtained.

RESULTS AND DISCUSSION

The present chromatographic system

This system was preceded by a slightly different instrumental set-up in which 250 × 1 mm I.D. glass-lined columns were used and carbon dioxide was delivered by an ISCO pump working under constant pressure [32]. The columns used were packed in-house as some purchased columns were unacceptable regarding efficiency and peak symmetry. However, as the selectivity with the preferred packing material was poor [32] and we encountered problems in making reproducible columns, the system was changed to one in which standard-size packed columns could be used. Also, an advantage is the less critical demand on the tubing and the connections compared with using small I.D. (50 μm) fused-silica tubing and microbore columns.

The weak point of this system is the regulation of the back-pressure. It was not possible to keep the back-pressure at the same value for all columns but as the percentage of modifier was of greater importance for the capacity factors than was the actual outlet pressure, and hence the density in the system, this was thought to be a minor problem at this stage of development. The back-pressure will be under control in the future [33,34]. Minor changes in modifier concentration affect the capacity factor more than do minor changes of pressure [23], although this does not exclude the possibility that the selectivity might change with varying density.

The mobile phase used throughout was carbon dioxide with methanol as the main modifier and triethylamine added as base. The presence of an amine or base in the modifier is commonly used in packed-column SFC [10,14,18,22,23,25,35,36] when the analytes contain aliphatic nitrogens and is in analogy with practice in liquid chromatography [9].

Selection of column support with suitable selectivity

The separation factors (α) relative to omeprazole for the investigated columns and substances are illustrated in Fig. 2. The actual capacity factors for omeprazole, and the inlet pressures and linear velocity of the mobile phase, are given in Table I. The columns were tested in duplicate except for the Si-60, dimethylamino and the diol columns. The last-mentioned type has been extensively investigated previously [32].

From Fig. 2 it is evident that the selectivity is not adequate with LiChrosorb RP-18 or Superspher Si-60 (bare silica). With the former support there is insufficient selectivity between omeprazole and its desmethoxylated analogue, and with bare silica the last-mentioned compound and methylated omeprazole co-elute (Fig. 2). With the diol support most compounds are close to omeprazole (Fig. 2). This is in agreement with previous results [32].

It is interesting to observe the reasonably good agreement between the aminopropylsilica columns from the three different suppliers, Macherey–Nagel, Merck and Eka Nobel. For this kind of support the order of elution is according to increasing polarity of the analytes. The elution order is illustrated with the chromatogram in Fig. 3. With dimethylamino-modified silica an increase in selectivity is found between omeprazole and the sulphide and the sulphone. Selectivity factors from columns run in duplicate agree reasonably well considering the absence of control of the back-pressure.

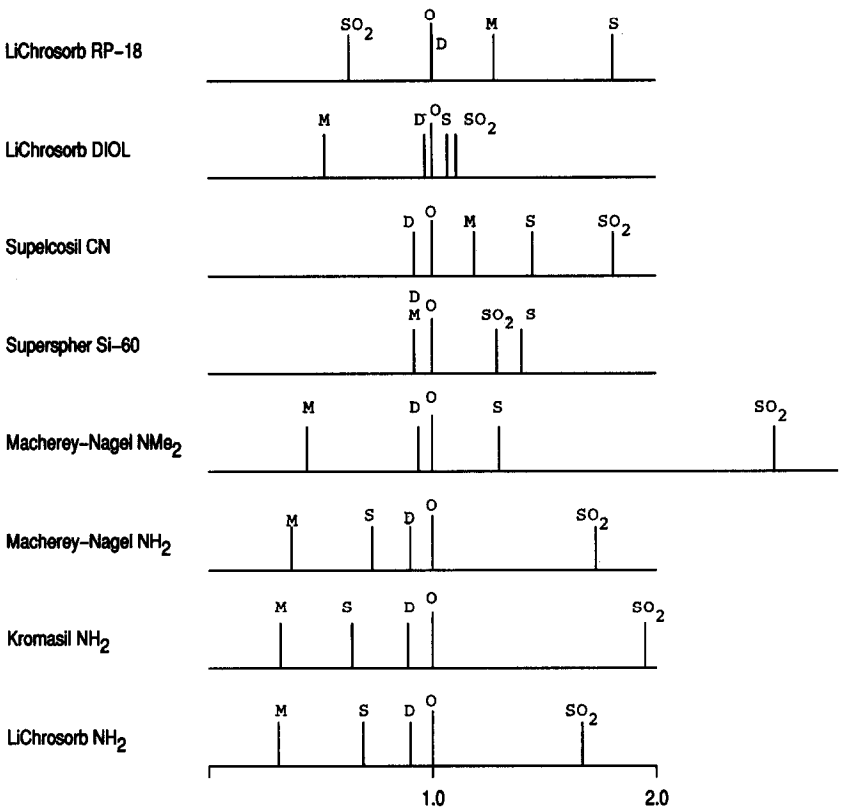

Fig. 2. Illustration of the selectivity factors relative to omeprazole with different column supports. Compounds: O = omeprazole; M = methylated omeprazole isomers (H 193/61); D = desmethoxyomeprazole (H 180/29); S = corresponding sulphide (H 168/22); SO_2 = corresponding sulphone (H 168/66) (*cf.*, Fig. 1). For conditions, see Experimental section and Table I.

The capacity factors for omeprazole show high values using bare silica and aminopropylsilica from Macherey–Nagel (Table I). In the former instance a stable equilibrium was first reached after about 6 h of continuous work. The capacity factors decreased by about 30% over this period. It is possible that a significant layer of adsorbed methanol acts as a stationary phase that retains the analytes to a larger extent than on, *e.g.*, RP-18 (Table I). With the amine column mentioned the high capacity factor may partly be due to a fairly low outlet pressure (Table I).

Selection of column support with the least residual activity

The asymmetry factors for omeprazole and its analogues were measured in order to obtain an indication of remaining active sites on the support. Most columns gave good symmetry for methylated omeprazole (Table I), which lacks the slightly acidic nitrogen in the imidazole ring (Fig. 1). However, with the diol column poor symmetry (*ca.* 2) was observed also for this compound (Table I). Although the peak shape is reasonable using RP-18 or Superspher silica, they do not have sufficient selectivity (see above). Of the columns tested, the aminopropylsilica columns from Merck and Eka Nobel showed the best overall symmetry for the compounds of interest (Table I). As the latter contains *ca.* 30% more packing material and the capacity factors are a slightly high, the former was preferred for testing with actual omeprazole samples. The chromatogram in Fig. 3 shows the separation and good peak symmetry obtained with this column. The greater inertness of the aminopropyl type of silica has been reported elsewhere [26,37] and is evident from other reports [23,25,27].

TABLE I

SELECTION OF SUITABLE COLUMN SUPPORTS: ASYMMETRY FACTORS AND CHROMATOGRAPHIC DETAILS

Pump flows, 1.20 ml/min CO_2 and 60 μl/min of modifier (1% TEA in methanol). For other details and conditions, see Experimental section. Structures in Fig. 1.

Column (support)	Asymmetry factors				k' (omeprazole)	Linear flow-rate (cm/s)	Inlet/outlet pressure (bar)
	Methylated omeprazole[a]	Omeprazole	Sulphide (H 168/22)	Sulphone (H 168/66)			
LiChrosorb RP-18	1.4	1.5	1.6	1.15	2.57	0.15	122/120
LiChrosorb Diol	2.1	2.1	2.8	2.2	5.56	0.13	273/269
Supelco CN	0.9	1.8	>4	1.2	5.06	0.16	95/96
Superspher Si-60	1.2	1.45	nm[b]	1.33	28.6	0.17	142/130
Macherey–Nagel $N(CH_3)_2$	1.22	1.68	nm	nm	7.23	0.14	126/126
Macherey–Nagel NH_2	1.0	nm	>2	>2	31.3	0.15	118/83
Kromasil NH_2	1.0	0.95	0.94	nm	14.6	0.13	119/105
LiChrosorb NH_2	0.9	1.22	1.37	1.44	9.13	0.15	175/nm

[a] Given for the second isomer of the pair.
[b] nm = Not measured.

Chromatographic analysis of omeprazole substance

Using the selected aminopropylsilica column, the possibility of determining omeprazole of different origins was tested. In order to reduce the time of analysis, the flow-rate and the percentage of modifier were increased. The last aspect is crucial, as the separation of omeprazole and its desmethoxylated analogue is easily destroyed with too much modifier. In Fig. 4 a successful separation within 10 min where minute amounts of possible contaminants had been added to omeprazole is shown. Regulatory requirements require that at least 0.1% of impu-

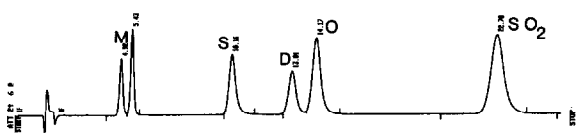

Fig. 3. Chromatogram of omeprazole and related compounds on LiChrosorb NH_2 using 1.2 ml/min of CO_2 and 60 μl/min of 1% TEA in methanol. Peaks as in Fig. 2. Inlet pressure, 175 bar.

TABLE II

COMPARISON OF LC AND SFC IN THE STUDY OF DEGRADATES FORMED FROM OMEPRAZOLE SODIUM UNDER ACCELERATED CONDITIONS

The degradates are given as a percentage of the omeprazole peaks, as the chromatographic pattern of the other degradates was not identical.

Compound	Sample A[a]		Sample B[a]	
	LC	SFC	LC	SFC
Omeprazole	100	100	100	100
Methylated omeprazole	3.66[b]	1.23, 2.39, 3.62	0.23[b]	0.07, 0.14, 0.21
Sulphide	nm[c]	0.35	nm[c]	nd[d]
Sulphone	2.02	2.33	3.00	2.62

[a] A is omeprazole sodium from storage at 50°C and B is from a rejected batch.
[b] The isomers of methylated omeprazole were not separated by LC [3].
[c] The sulphide was not measured by LC; retention time about 25 min [3].
[d] nd = Not detected.

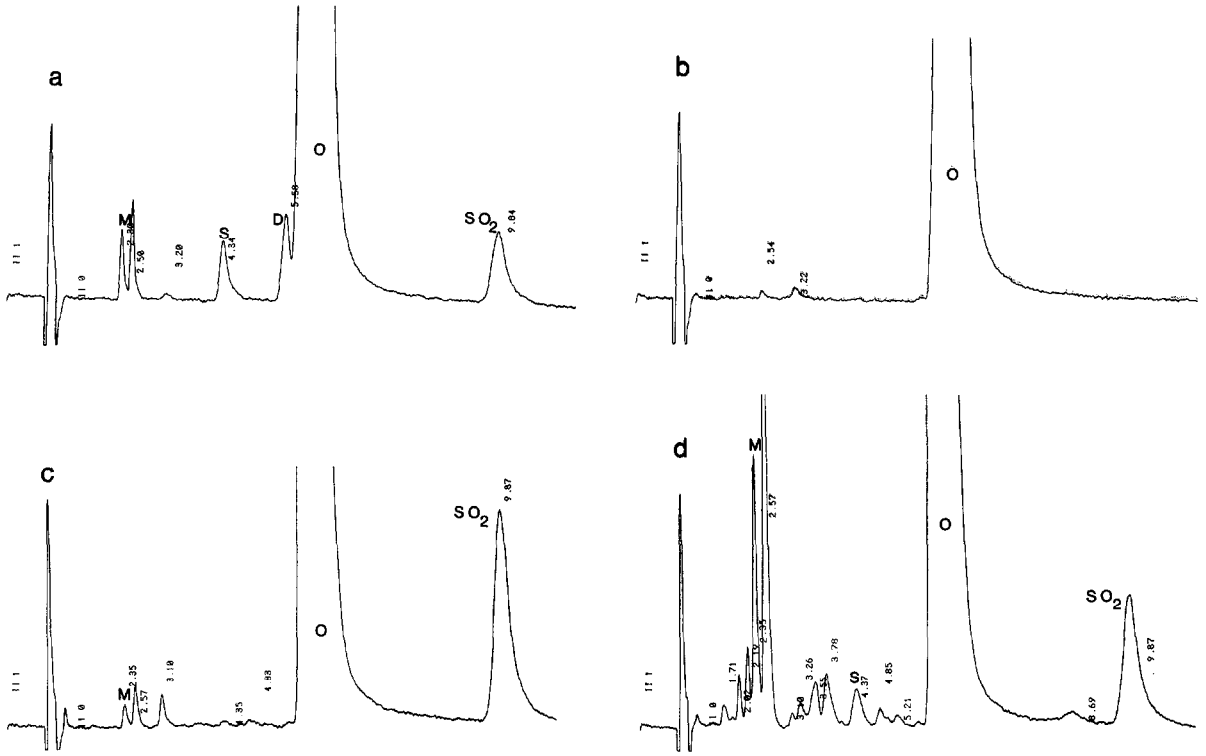

Fig. 4. Chromatogram of different batches of omeprazole substance, *ca.* 4 mg/ml in dichloromethane. Conditions: CO_2 2.0 ml/min and 1% TEA in methanol 120 μl/min; column, LiChrosorb NH_2 at 40°C; inlet pressure, 183 bar; outlet pressure, 175 bars; linear velocity, 0.27 cm/s. Peaks as in Fig. 2. (a) With possible impurities added (0.4–0.6%, w/w); (b) pure omeprazole substance; (c) omeprazole sodium from a rejected batch; (d) omeprazole sodium that had been stored at 50°C.

rities can be determined. It should also be noted that the isomers of methylated omeprazole have different ε values. A corresponding chromatogram with pure omeprazole is given in Fig. 4b and here the sum of extraneous peaks constitutes less than 0.1 area-% at 300 nm. Fig. 4c and d show chromatograms from omeprazole sodium of different origin. In Fig. 4c the substance had been freeze-dried but rejected after inspection after storage for 3 months at ambient temperature. The chromatogram in Fig. 4d is for omeprazole sodium that had been stored at 50°C for a certain period of time. Here more of the methylated omeprazole isomers are formed than shown in Fig. 4c. Analysis by liquid chromatography gave the same patterns, as can be seen from Table II. However, the liquid chromatographic method cannot discriminate between the isomers of methylated omeprazole although they elute after about 12 min [3]. As the sulphide requires 25 min for elution, the SFC system is about 2.5 times faster and has a higher degree of selectivity.

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