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Chromatographic purification of some 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors ☆

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Abstract

The purification of pravastatin, simvastatin and lovastatin in the sodium salt or lactone form and of mevastatin in the lactone form by reversed-phase displacement chromatography is presented. The mobile phases consisted of water or mixtures of water–methanol and water–acetonitrile. Six different displacers were successfully used. Up to 0.14 g of raw sample per gram of stationary phase was loaded on a column packed with silica-based octadecyl phase. Crude substances from 85 to 88% chromatographic purity were purified and at least 99.5% purity was achieved.

Keywords: Displacement chromatography; Preparative chromatography; Pharmaceutical analysis; Enzyme inhibitors; Pravastatin; Simvastatin; Lovastatin; Mevastatin

1. Introduction

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors lovastatin, pravastatin, simvastatin, mevastatin are cholesterol lowering agents and are widely used for treatment of hypercholesterolemia [1–3] when taken on a long-term basis.

The purity of drug substance is an important factor in the manufacture of a safe and effective pharmaceutical. Preparative high-performance liquid chromatography (P-HPLC) is employed in separation and purification of complex organic molecules [4]. Although overloaded elution chromatography is mostly used because of its ease of operation, displacement chromatography has some advantages [5–7].

Displacement chromatography [8–10] is based on the competition of the components of the sample feed in the column for the stationary phase. Individual components of the sample displace one another. The displacer having the highest affinity for the stationary phase and travelling behind the sample feed along the column drives the separation. Under appropriate conditions, a displacement train is formed containing the feed components as adjacent bands, all moving with the velocity of the displacer front. In comparison to elution chromatography larger feed can be separated on a given column with the purified components recovered at higher concentrations [11]. A variety of applications of displacement chromatography have been published in the literature dealing with ‘small’ organic compounds [5,9,12–16], peptides [6,17–21], proteins

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[22–26] and oligonucleotides [27,28]. However, displacement chromatographic purification of lovastatin, pravastatin, simvastatin and mevastatin has not previously been described.

In our work reversed-phase displacement chromatographic purification of pravastatin, simvastatin, lovastatin in the sodium salt or lactone form and of mevastatin in the lactone form is presented. Raw samples from down stream process were purified having chromatographic purity from 85 to 88% with several impurities up to 8%. Different displacers, mobile phases and reversed-stationary phases were used.

2. Experimental

2.1. Materials

Crude lovastatin, pravastatin, simvastatin sodium salts or lactons and mevastatin lactone were from Lek (Ljubljana, Slovenia). Stationary phases Lichrospher 100 C_{18} 12 μm was from Merck (Darmstadt, Germany) and Grom-sil ODS 11 μm HE from GROM Analytik+HPLC (Herrenberg, Germany) were slurry packed in 200×10 mm columns in our laboratory. HPLC columns: Betasil C_{18} 3 μm (50×4.6 mm) was from Keystone Scientific (Bellefonte, PA, USA), Spherisorb ODS2 3 μm (50×4.6 mm) and Hypersil ODS 3 μm (125×4.6 mm) were from HPLC Technology (Welliongton House, UK). Methanol, acetonitrile, 85% phosphoric acid, ammonium hydrogencarbonate, acetic acid, triethylamine and diethylphtalate (DEP) were from Merck (Darmstadt, Germany). Diethyleneglycol monobutyl ether (DEGBE), diethyleneglycol dibutyl ether (DEGDBE), diethyleneglycol mono-*n*-hexyl ether (DEGHE), decanoic acid (DA) and tetrakis-(decyl)ammonium bromide (TDAB) were from Aldrich (Gillingham, UK). Deionized water of at least 18 MΩ was purified by an Elga UHQ (High Wycombe, UK) apparatus.

2.2. Displacement chromatography operation

The displacement chromatography was performed using a Gilson (Villiers le Bel, France) HPLC system consisting of four pumps (model 306) with 25 SC pump heads, UV detector (model 117) equipped with 0.05 mm preparative flow cell, dynamic mixer (model 811C) with 1.5 ml mixing chamber, manometric module (model 806) and automatic fraction collector (model 201). The system was controlled and data were collected with a Gilson 715 system controller software.

The column was equilibrated with the mobile phase. The sample was dissolved in mobile phase and loaded on the column, followed by the displacer solution. Absorbance was measured at 260 nm and 0.5 ml fractions were collected with an initial increase in the absorbance. After the elution of the main peak, displacer was washed from the column with 50 ml of methanol. Mobile phases, columns, sample loads, displacers type, concentrations and flow-rates are summarised in Table 1. During the column equilibration, sample loading and column regeneration the flow-rate was 10 ml/min. The obtained fractions were analysed by a fast HPLC method. Fractions with chromatographic purity equal to or higher than 99.5% were pooled.

2.3. Fractions analysis

The HPLC analyses of fractions were carried out on a Hewlett-Packard (HP) 1100 (Waldbronn, Germany) chromatographic system consisting of a quaternary pump, an autosampler, and a diode array UV detection (DAD) system. The system was controlled and data were collected with HP Chemstation system controller software.

For the analysis of displacement fractions of pravastatin, lovastatin and simvastatin, all in the sodium salt form, mobile phases of 10 m*M* ammonium hydrogen carbonate and 15, 40 and 45% acetonitrile, respectively were used. The column was Spherisorb ODS2 3 μm (50×4.6 mm). Displacement fractions of pravastatin, lovastatin, simvastatin and mevastatin, all in the lactone form, were analysed using mobile phases consisting of 0.05% phosphoric acid and 20, 60, 68 and 55% acetonitrile, respectively on Betasil C_{18} 3 μm (50×4.6 mm) column. The UV detection was at 235 nm.

This method was also used for the determination of the concentration of the main component in pooled fractions.

Table 1
Chromatographic conditions and results of displacement chromatography of different HMG-CoA reductase inhibitors[a]

	Mobile phase	Displacer type	Displacer conc. % (m/v)	Column	Flow rate (ml/min)	Sample load (mg)	Chromatographic purity (%)		Conc. (mg/ml) in pooled frac.	Amount of purified product (mg)	Run time (min)
							Load	Pooled frac.			
Pravastatin sodium	Water	DEGBE	7	A	3	1000	88	99.8	130	715	30
	30% Methanol	DEGBE	7	A	3	600	88	99.8	100	400	25
Pravastatin lactone	30% Methanol	DEGHE	5	B	4.5	300	85	99.8	50	175	36
	35% Acetonitrile	DEGDBE	1	B	4.5	300	85	99.8	15	140	60
	35% Acetonitrile	DEP	0.85	B	4.5	300	85	99.8	15	135	55
Simvastatin sodium	14% Methanol	DEGHE	6.7	A	4.5	500	87	99.8	80	280	30
	14% Methanol	DEGHE	14	A	4.5	500	87	99.8	170	255	23
Simvastatin lactone	70% Methanol	DA	3	A	4.5	700	87	99.7	65	490	28
	60% Acetonrile	TDAB	2	A	4.5	500	87	99.8	50	300	30
Lovastatin sodium	14% Methanol	DEGHE	6.7	A	1	400	87	99.8	80	200	75
Lovastatin lactone	75% Methanol	DA	4.5	A	6	500	87	99.9	65	195	20
Mevastatin lactone	70% Methanol	DA	4.5	A	6	500	85	99.8	65	160	22

[a] Columns; A: Grom-Sil ODS4 HE (11 μm, 200×10 mm) B: LiChrospher 100 RP18 (12 μm, 200×10 mm).

2.4. Sample feed and pooled fractions analysis

The same equipment was used as for fraction analysis. Mobile phase A consisted of 0.1% of acetic acid and 0.1% of triethylamine in water and methanol was used. For mobile phase B 0.1% of acetic acid and 0.1% of triethylamine in methanol was used. The content of methanol in the mobile phase A was adjusted to obtain the retention factor of the main component to about 9, then linear gradient to mobile phase B in 9 min was applied. The UV detection was at 235 nm.

2.5. Determination of displacers DEGBE and DA in fractions

GC–MS was carried out with a model 3300 gas chromatograph from Varian (Sugar Land, TX, USA) and with a SSQ 700 mass spectrometer from Finnigan MAT (San Jose, CA, USA) were used in the determination of DEGBE and DA in fractions.

DEGBE analyses were carried out on a fused-silica column (25 m×0.25 mm I.D.) coated with CP-SIL 5CB (film thickness 0.25 μm) from Chrompack (Middelburg, Netherlands). A temperature gradient from 90°C to 170°C at 20°C/min was used and the mass spectrometer scanning range was from m/z 46 to 200 in 0.3 s. Injector and transfer line temperatures were 300°C.

DA was determined on a fused-silica column (25 m×0.25 mm I.D.) coated with CP WAX 58 FFAP-CP (film thickness 0.2 μm) from Chrompack. The temperature gradient was from 50°C to 250°C at 20°C/min and the mass spectrometer was set to monitor at m/z 60. Injector and transfer line temperatures were 250°C.

3. Results and discussion

The aim of our work was to investigate the possibilities of purification of some HMG-CoA reductase inhibitors with displacement chromatography on reversed-stationary phase to achieve chromatographic purity of the product higher than 99.5%.

For the selection of the appropriate displacer the isotherms of all involved components of the feed and displacer should be known. With complex samples it is impossible to measure the isotherms of all the components of the feed because they are normally not available. Therefore, the selection of the displacer by trial and error is inevitable [9].

To evaluate the displacement separation, the chromatographic purity and the concentration of the main component in the eluent were monitored. The displacement chromatogram was fractionated and the fractions were analysed with appropriate fast HPLC methods.

For pravastatin sodium displacement chromatography DEGBE was used as the displacer (Fig. 1).

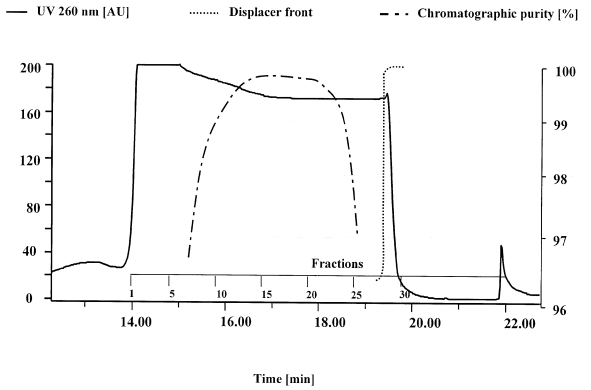

Fig. 1. Displacement chromatogram of pravastatin sodium on Grom-sil ODS (11 μm, 200×10 mm) column. Mobile phase, water; displacer, 7% DEGBE in mobile phase; flow-rate, 3 ml/min; feed, 1 g of raw pravastatin sodium in mobile phase.

The signal of the UV detector is saturated during the elution of the first five fractions. The chromatographic purity of the fractions reaches nearly 100%. The doted line represents the displacer front, the units are arbitrary. The displacer front is very sharp and no displacer is present in the pooled fractions. Analysis of fractions 4 and 15 of pravastatin sodium are shown in Fig. 2. A very high load, 1 g, of crude pravastatin sodium on the column was achieved.

For all displacement experiments the conditions and results (chromatographic purity and concentrations in pooled fractions, amount of purified products and run times) are summarised in Table 1.

Different gradient HPLC methods for the determination of the chromatographic purity of the sample load and the pooled fractions were used for different sample types. Fig. 3 shows the chromatograms of the sample load and pooled fractions of pravastatin sodium. A chromatographic purity of 99.83% for pravastatin sodium was achieved.

In the case of pravastatin lactone displacers DEGHE, DEGDBE and DEP were used. Low solubility of DEGDBE and DEP in the mobile phase resulted in longer separation times and lower final concentrations of the pooled fractions.

Simvastatin sodium was displaced with DEGHE. Two displacer concentrations were used (6.7 and 14%) and high concentrations in pooled fractions (80 and 170 mg/ml) were achieved.

DA and TDAB were used in displacement of simvastatin lactone. To achieve sufficient solubility of displacer DA and simvastatin lactone mobile phase with high content of organic modifier (70% methanol or 60% acetonitrile) was used.

In the displacement chromatogram of simvastatin lactone (Fig. 4) a peak after the main displacement peak at the retention time of approximately 22.5 min is present. The impurities were not displaced by DA but eluted during the regeneration of the column with methanol. Displacer appears in fraction 20 (represented by the dotted line). The chromatographic purity of fractions 13–25 was equal to or higher than 99.9%.

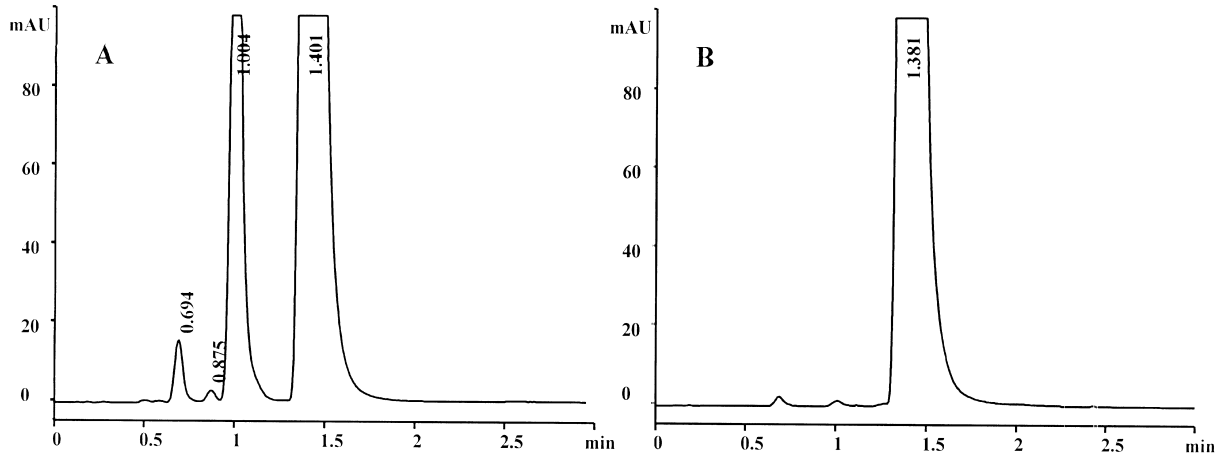

Fig. 2. Analysis of pravastatin sodium displacement fractions 4 (A) and 15 (B) on Spherisorb ODS2 (3 μm, 50×4.6 mm) column. Mobil phase, 10 m*M* $(NH_4)HCO_3$, 15% acetonitrile; flow-rate, 2 ml/min; detection, 235 nm.

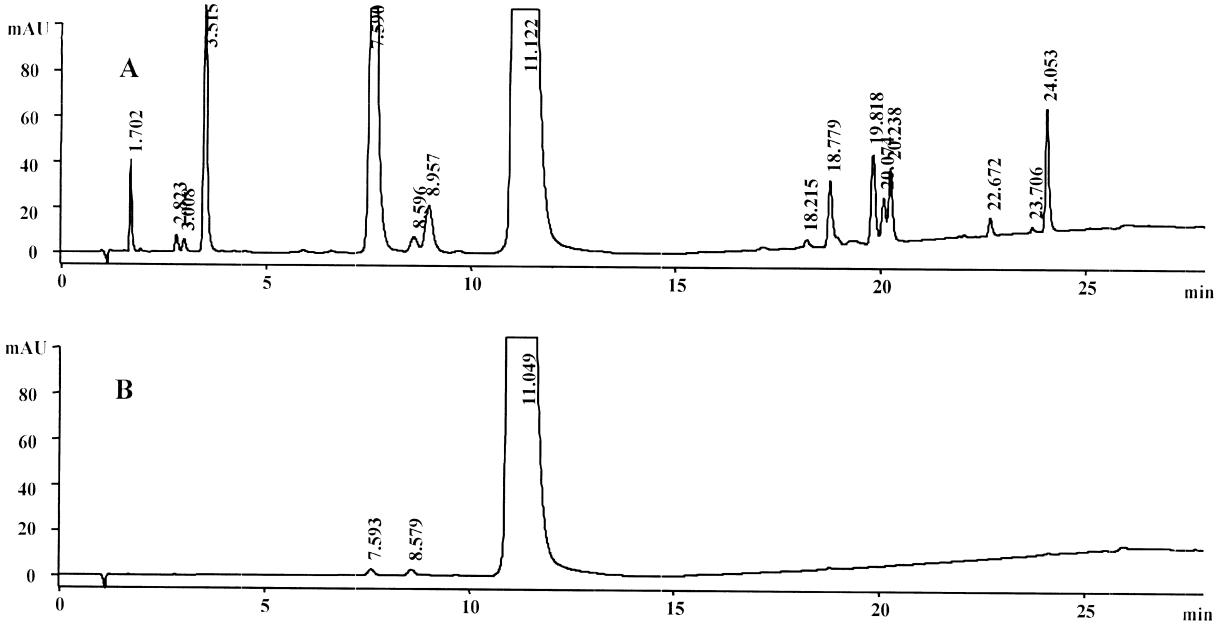

Fig. 3. Gradient analysis of pravastatin sodium, feed (A) and pooled fractions (B) on Hypersil ODS (3 μm, 125×4.6 mm) column. Mobile phase A, 0.1% triethylamine, 0.1% acetic acid, 45% methanol; mobile phase B, 0.1% triethylamine, 0.1% acetic acid in methanol; gradient, 13 min 0% B then in 9 min to 100% B; flow-rate, 1.2 ml/min; detection, 235 nm.

In Fig. 5 the chromatograms of fractions 4 and 12 of simvastatin lactone displacement purification are shown.

Displacer DEGHE was also used for lovastatin sodium purification with expected results. For purification of lovastatin lactone and mevastatin lactone the same displacer (DA) and similar conditions were used as for simvastatin lactone.

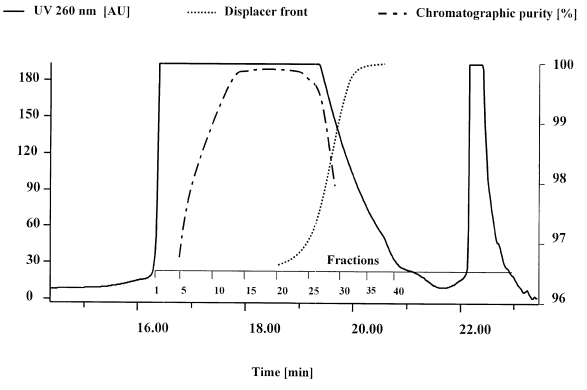

Fig. 4. Displacement chromatogram of simvastatin lactone on Grom-sil ODS (11 μm, 200×10 mm) column. Mobile phase, 70% methanol; displacer, 3% DA in mobile phase; flow-rate, 4.5 ml/min; feed, 0.7 g of raw simvastatin lactone in mobile phase.

The selection of a displacer is the most critical step in developing an efficient displacement chromatography separation [9]. The displacer has to bind to the stationary phase stronger than the component of the sample of interest. The solubility of the displacer has to be high enough to avoid too long displacement process. In the same time the elution strength of the mobile phase has to be low enough to avoid elution of the sample. In a selection of suitable displacement conditions a compromise between the elution strength of the mobile phase and the solubility of the displacer and the sample is often needed.

Displacement method optimisation, the effect of the stationary phase, flow-rate, load and of displacer type are not the matter of this manuscript and will be published later (R. Grahek, in preparation).

4. Conclusion

The high level of purity required for pharmaceutical products has dramatically increased the

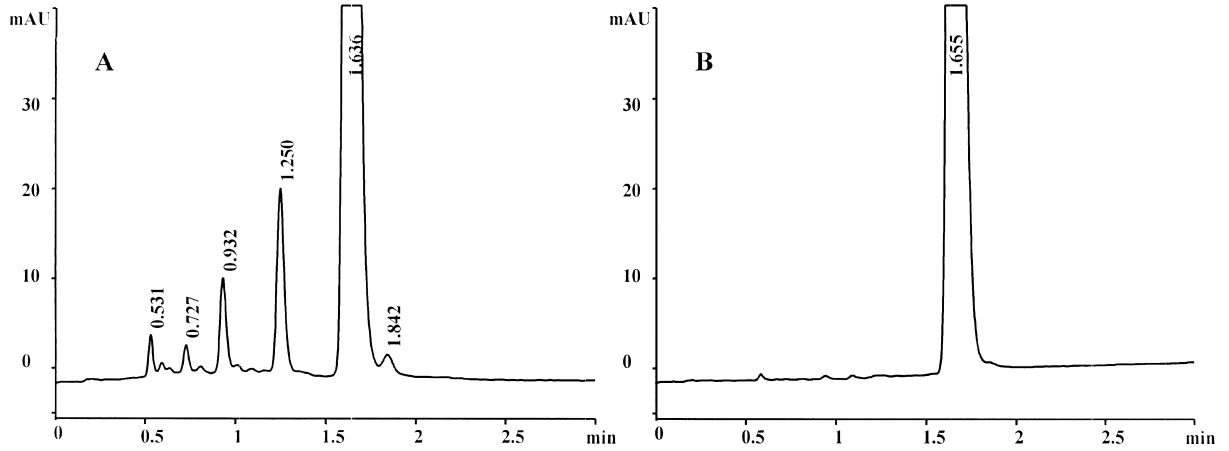

Fig. 5. Analysis of simvastatin lactone displacement fractions 4 (A) and 12 (B) on Betasil C_{18} (3 μm, 50×4.6 mm) column. Mobile phase, 0.05% phosphoric acid, 66% acetonitrile; flow-rate, 2 ml/min; detection, 235 nm.

importance of preparative liquid chromatography in purification and isolation processes on industrial scale. Displacement chromatography of lovastatin, pravastatin, and simvastatin in the sodium salt or lactone form and of mevastatin in the lactone form has the potential for the economical large-scale purification. It has been demonstrated that is possible to obtain a product of chromatographic purity higher than 99.5% with a feed load up to 0.14 g per 1 g of stationary phase. The pure product can be obtained in highly concentrated solutions. The efficient use of six displacers was shown.

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