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Potential of packed column supercritical fluid chromatography for the separation of metoprolol from closely related compounds ☆

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Abstract

Using the present SFC method metoprolol can be separated from 12 analogues and related compounds within 12 min. It is also possible to detect most of these analogues at the 0.1% (w/w) level when added to the pure drug substance. The method is based on SFC with modified carbon dioxide as the mobile phase and a packed 4 mm I.D. diol-modified silica column. The mobile phase modifier is 10% (v/v) methanol containing 0.35 *M* of acetic acid and 0.07 *M* of triethylamine. UV detection is performed at 273 nm. The main advantage with the described method over the currently used reversed-phase liquid chromatographic method is the different selectivity, i.e., it is possible to separate and detect isomers that elute close to the metoprolol peak in currently used liquid chromatographic systems.

Keywords: Packed column supercritical fluid chromatography; Metoprolol; β-Blockers

1. Introduction

Metoprolol (Fig. 1) is a β-adrenoreceptor blocking agent widely used as a drug in the treatment of hypertension and the prevention of angina. The analytical methods of choice today, for bulk substance purity and content uniformity analysis, are based on reversed-phase liquid chromatography (LC) with alkyl silyl modified silica as support followed by UV detection [1]. The mobile phase can be either heptane sulphonic acid, or triethyl ammonium acetate [1] in aqueous acetonitrile at low pH.

Metoprolol and several analogues can be chromatographed in their native form on fused-silica capillary columns with flame-ionization detection using supercritical fluid carbon dioxide or nitrous oxide as the mobile phase [2]. This technique is hampered by the acidic nature of the silica columns and the problem of detecting below 1% of analogues while at the same time keeping the main peak metoprolol from overloading the stationary phase due to the small dimensions and film thickness of these columns.

During the last few years packed column supercritical fluid chromatography (SFC) has found renewed interest [3,4]. The main attraction is the shorter time for analysis, the adequate sample capacity available and the selectivity that more resembles that of normal-phase LC. Furthermore the handling of instrument and columns is much less complicated than in capillary SFC. Most LC type of columns can be used without any special precaution. The technique has been used for the separation of

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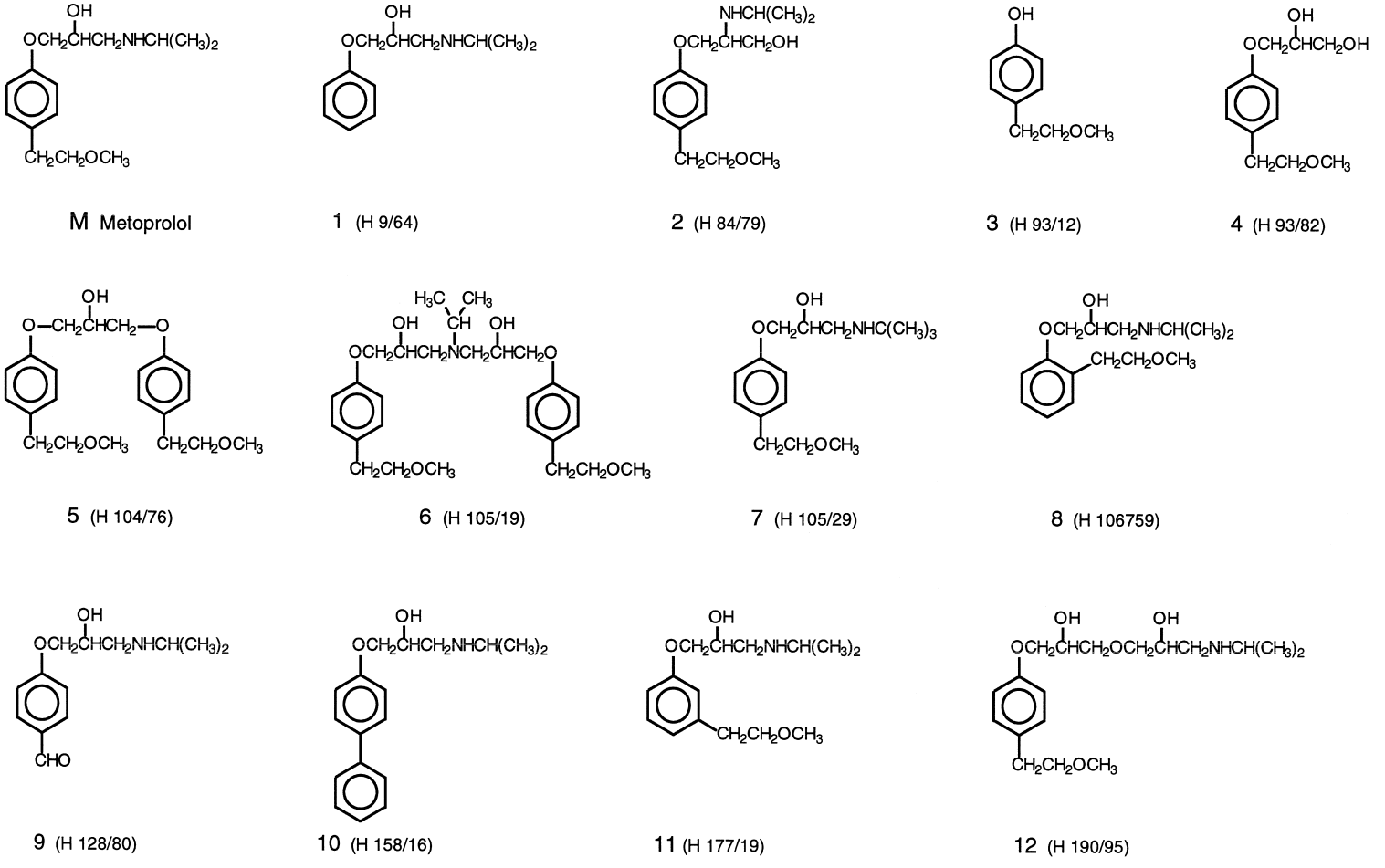

Fig. 1. The structure of metoprolol and related compounds. Company codes given in parentheses.

propranolol [5] and several different β-adreno-receptor blocking drugs [6]. Concerning such drugs many publications in the more recent literature have shown the possibility to use both traditional, and new straight phase enantioselective columns, for the determination of the individual enantiomers [7–11], also within a very short time [9,11,12]. The *R* enantiomer of propranolol was separated from the *S* enantiomer and quantified at the 0.1% level in 5 min [12]. However, little interest has been devoted to more traditional pharmaceutical problems such as the separation of closely related compounds in order to characterize the purity of the drug substance in question, or to determine the content uniformity of finished drug products. Even if such a method would not be likely to replace existing methods, that have proven their capability through long time use, the knowledge gained can be of importance for new methods of other candidate drugs.

The aim of the present work was to evaluate the merits of SFC with packed columns for the determination of metoprolol and analogues in bulk drug substance and the finished tablet product. A comparison with methods based on LC (straight and reversed-phase), gas chromatography, thin-layer chromatography and capillary electrophoresis will be dealt with in a separate paper [13].

2. Experimental

2.1. Instrumentation

The SFC instrument was a Hewlett-Packard G1205A (Little Falls Site, DE, USA) equipped with dual pumps, a flow meter for the modifier, a variable-wavelength UV detector and an autosampler. The instrument was controlled by the HP ChemStation software. Normal conditions were as follows: carbon dioxide (3.5 grade from AGA, Lidingö, Sweden) with 10% (v/v) methanol (containing the appropriate additive), flow-rate 2.0 ml/min, back pressure 150 bar, column oven 40°C and the detector set to monitor λ 273 nm. The columns investigated were 125×4 mm I.D. LiChrosorb aminopropyl silica and LiChrospher 100 diol 5 μm silica (E. Merck, Darmstadt, Germany). For work with three diol columns, connected in series, the other two contained LiChrosorb silica. The samples were dissolved in dichloromethane or methanol as indicated and the volume injected was 5 μl.

2.2. Chemicals and reagents

Metoprolol tartrate, metoprolol succinate, and the analogues of metoprolol were supplied by the Department of Medicinal Chemistry, Astra Hässle, Mölndal Sweden. Their structures are shown in Fig. 1. Solvents were of analytical-reagent grade as were acetic and citric acids. Triethylamine had been glass distilled.

2.3. Methods

Metoprolol tartrate and analogues, generally hydrochlorides were dissolved in dichloromethane before analysis. Metoprolol succinate was dissolved in methanol. Solutions of 0.1% (w/w) of analogues relative to metoprolol were calculated as free bases.

Metoprolol succinate in tablets was isolated as follows. One tablet of 47.5 mg was crushed in a mortar, mixed with 2.0 ml of methanol, after 15 min of sonication filtered through a glass microfibre filter (Whatman 934AH, Maidstone, UK).

3. Results and discussion

3.1. Choice of chromatographic system

Earlier studies on the SFC of omeprazole and analogues showed that good selectivity could be obtained by selection of a suitable chromatographic support [3]. For basic compounds it is usually necessary to have an aliphatic amine present in the mobile phase [14–17] and this has been the rule in most papers published on the analysis of amino alcohols by SFC [5–12]. In preliminary experiments using Si 60 bare silica the wide difference in retention between early and late eluting compounds demanded a modifier gradient. This approach was rejected. Instead silica modified with aminopropyl- or diol-groups were selected for further investigations. Using 10% of methanol as modifier containing 0.07 *M* of triethylamine the selectivity was in most instances good on both of these supports (Table 1).

Table 1
Selectivity factors (α[a]) for metoprolol and analogues with different support and mobile phase modifiers in the carbon dioxide

No. in Fig. 1	Aminopropyl-silica 0.07 *M* TEA	Diol silica 0.07 *M* TEA	Diol silica 0.07 *M* TEA/0.35 *M* HAc	Important feature of compound
5	0.11	0.28	0.19	Phenol
3	0.10	0.26	0.22	Dimer, ether
6	0.22	0.43	0.54	Dimer, amine
4	0.31	0.54	0.41	Glycol
2	0.49	0.74	0.68	Primary alcohol
7	0.78	0.81	0.75	Tertbutyl
8	0.79	0.84	0.82	*Ortho* side-chain
11	0.94	0.95	0.95	*Meta* side-chain
1	0.97	1.00	0.89	Unsubstituted ring
M	1.00	1.00	1.00	*Para* side-chain
10	1.71	1.57	1.57	Biphenyl
9	n.m.[b]	n.m.	1.69	Aldehyde
12	n.m.	n.m.	2.24	Prolonged

[a] $\alpha = k'_2/k'_{\text{metoprolol}}$.
[b] n.m. = Not measured.

Previous results and experience within our laboratory have shown that the continuous use of a basic additives can degrade the silica support but also that basic compounds could be chromatographed in the presence of acidic additives [10,17,18]. Therefore an excess of an acid relative to the amine was added in order to promote the stability of the columns. For the diol column there was only a marginal change in the selectivity (Table 1) whereas the retention times were shortened.

For some of these metoprolol analogues the selectivity factor was close to 1.00 (Table 1). In actual samples with an excess of the main compound this can be a problem but it can be solved as will be shown below. The selectivity in this packed column SFC system is surprisingly good for certain compounds compared to reversed-phase LC such as compounds 2 and 12 (Fig. 1), i.e., metoprolol extended with a hydroxypropyloxy moiety, are both separated with a high selectivity (Table 1) whereas in reversed-phase LC they elute close to metoprolol [19]. Interestingly enough only one peak is observed for this compound in our system although it has two stereogenic carbons which should in fact give two peaks. This compound is heavily retained (Table 1).

The chromatogram in Fig. 2 shows metoprolol and 10 possible analogues in the same run. Within about 10 min the 10 related compounds could be separated, most of them with a shorter retention than metoprolol itself. The resolution is not at baseline between some of the components in the region just before the metoprolol peak (Fig. 2). On the other hand it is not very likely that all analogues are present at the same time in an unknown sample of metoprolol bulk substance. Information on which compounds are potential impurities in metoprolol tartrate can be found in the European Pharmacopoeia [1].

Since it was not possible with the initial column and conditions to separate the analogue with the side-chain in 3-position (*meta*, compound 11 in Fig. 1) at the 0.1% (w/w) level we investigated the possibility to use three columns in series since with carbon dioxide the pressure drop can be expected to be low, as has been shown in other works [20,21]. A chromatogram from such a separation is shown in Fig. 3. Though the chromatographic peak is small, this analogue is clearly distinguished from the metoprolol peak. The pressure drop in this case was only about 90 bar over the three columns and the plate number above 8000 as compared to about 4000 for a single one, calculated from injections of diluted solutions of metoprolol.

3.2. Metoprolol tartrate and succinate bulk substance

Fig. 4a shows a chromatogram of metoprolol

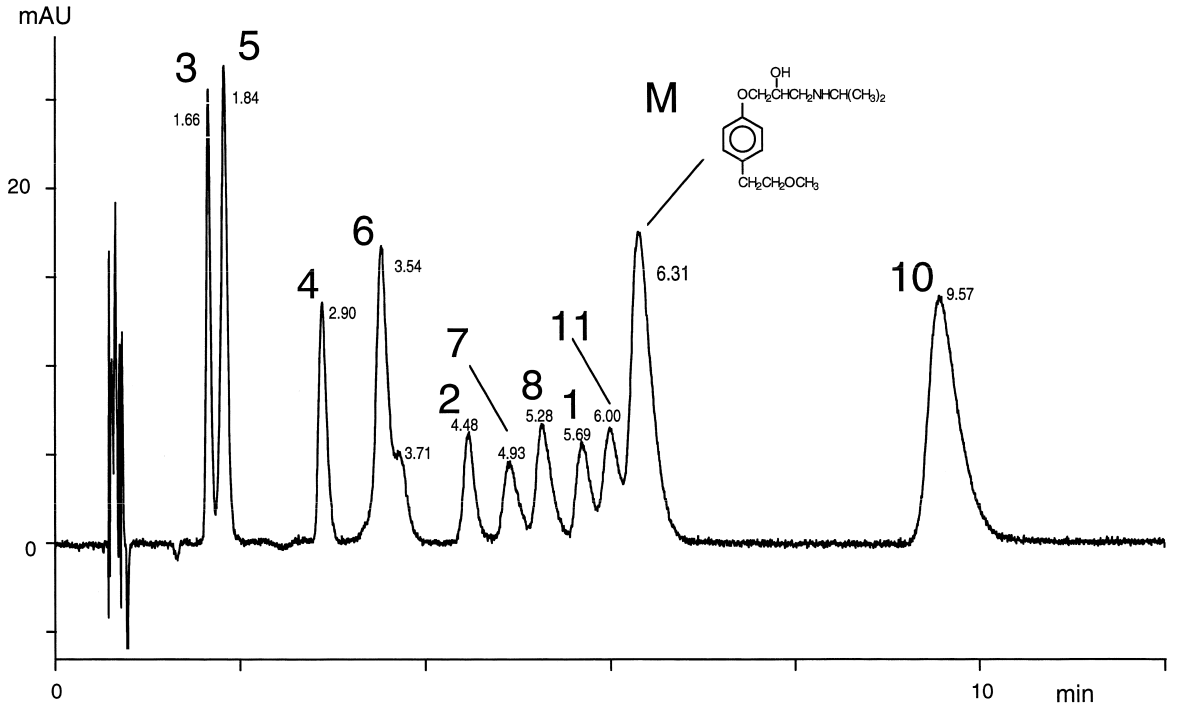

Fig. 2. Packed-column SFC–UV (273 nm) of metoprolol and related compounds. For more detailed structures see Fig. 1 and for conditions see Experimental.

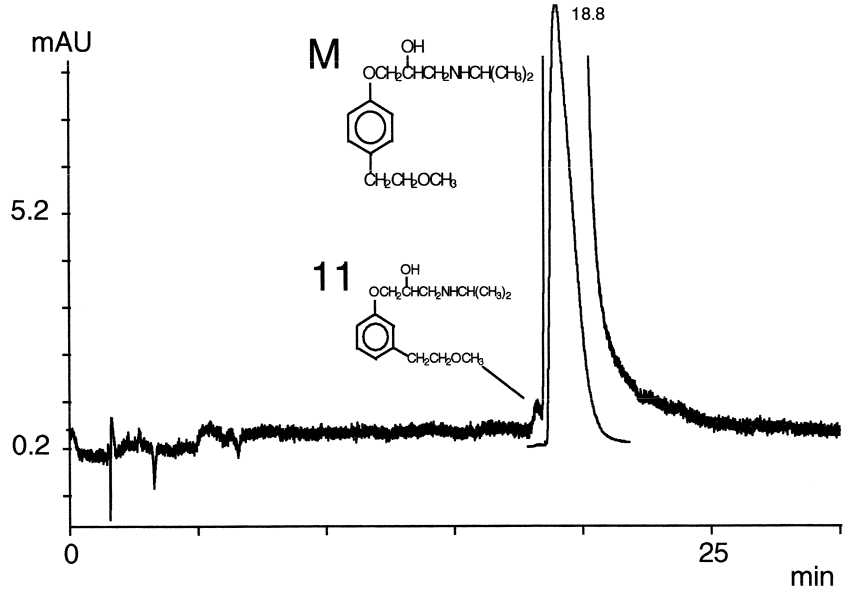

Fig. 3. Packed-column SFC–UV (273 nm) of metoprolol and its meta analogue (0.1%, w/w, added to metoprolol reference substance) using three diol columns in series. Conditions in Experimental. Inserted peak metoprolol, apex at 164 milli absorbance units.

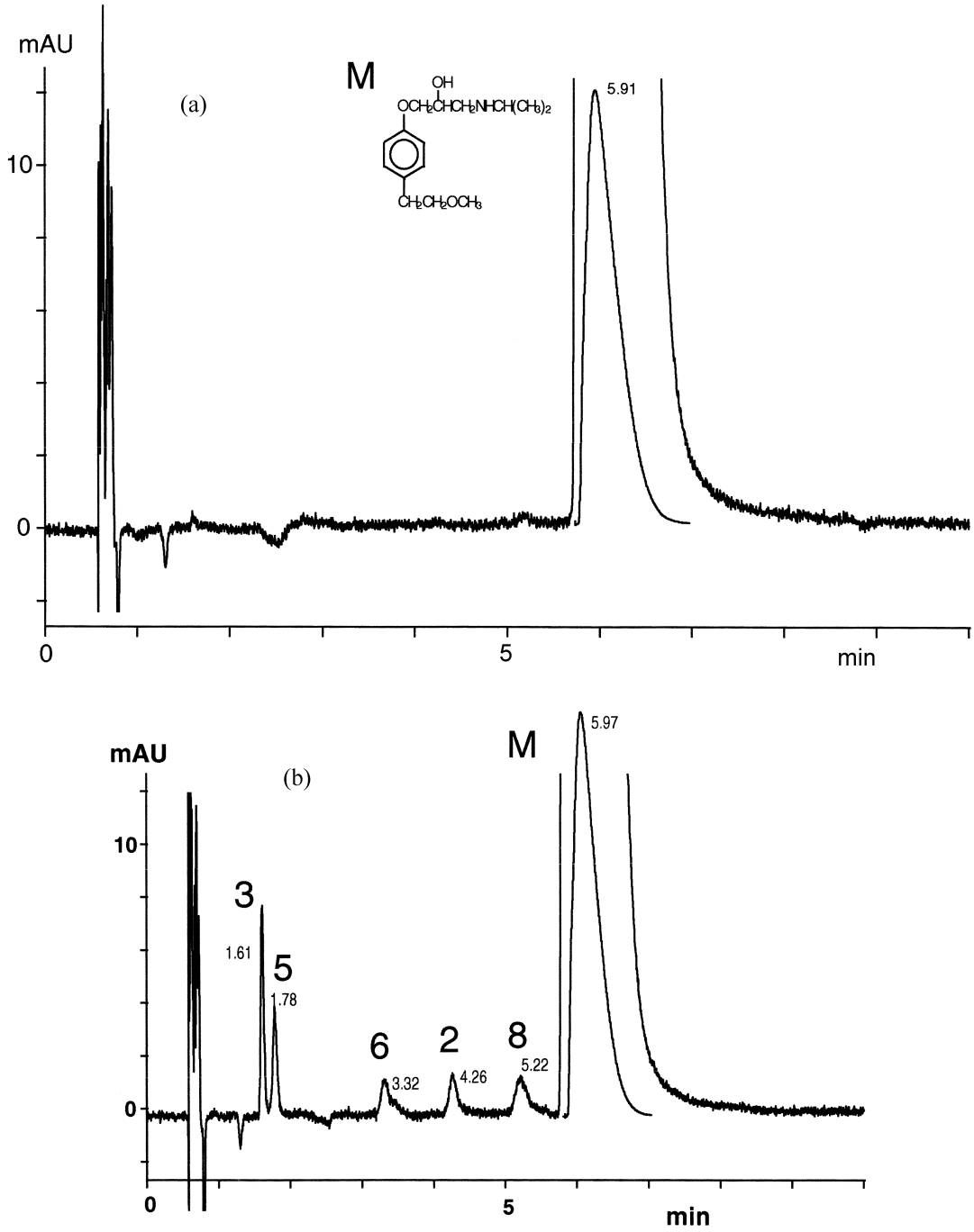

Fig. 4. Packed-column SFC–UV (273 nm) of metoprolol tartrate bulk substance. (a) Metoprolol tartrate bulk substance and (b) five analogues added to 0.1% (w/w) level. Conditions: see Experimental. Inserted peak metoprolol, apex at 440 and 410 milli absorbance units, respectively.

tartrate working standard substance. The metoprolol peak is large and has broadened to some extent. No extraneous peaks were observed in the region of interest. In the next chromatogram, Fig. 4b, the solution from Fig. 4a has been spiked with five analogues to the 0.1% (w/w) level. These analogues of metoprolol are clearly separated and detected at this level, which has been set by regulatory authorities. They were chosen since they are difficult to separate by reversed-phase LC.

As mentioned above the metoprolol peak has broadened. Still, it is within the linear range of the UV detector as can be seen from the standard curve constructed after chromatographic analysis of a range of concentrations of metoprolol tartrate in the range 80 μg/ml to 24.7 mg/ml including blanks (eight data points; $n=2$). The equation of the curve was $y=190.7x+5.37$, correlation coefficient 1.000. The autosampler gave some carry-over and thus the curve was not through the origin. Closer analysis of the peak width from the chromatograms at each level shows that the peak becomes wider when 20 μg or more is injected on the column. The retention time slightly decreased during this experiment, e.g., from 6.4 to 5.9 min when the peak broadens.

The integrated areas were in the range 0.1–0.2% of the metoprolol peak. Based on the chromatogram in Fig. 4b and three-times the baseline noise the detection limit of compounds 2, 6 and 8 is estimated to be 0.1% (w/w). For the early eluting peaks, compounds 3 and 5 it is about 0.02 and 0.04% (w/w), respectively using the present conditions.

In contrast to the tartrate salt the metoprolol succinate salt is not soluble to 20 mg/ml with dichloromethane as solvent. Instead methanol was used as solvent and this solvent had no detrimental effect on the peak width, although its higher polarity and solvating power as compared to dichloromethane. Apparently the mobile phase is sufficiently polar so that the polarity is little changed by the relatively small volume injected. Chromatograms after isolation of metoprolol from metoprolol succinate tablets revealed no interfering compounds from excipients. The yield of metoprolol was not investigated in detail since the volatility of the solvent reduced the volume of the sample to some extent. For content uniformity studies larger volumes are recommended and/or elimination of fines by centrifugation instead of filtration or loading the loop through a filtering device.

4. Conclusions

The merits of packed column SFC in the analysis of closely related impurities are the following: (a) the separation times are in general shorter than in most other separation methods. (b) The separation efficiency is in general high with the extra bonus of coupling several columns in series to increase the number of theoretical plates without losing much in column performance. (c) The loading is no problem as the packed version has high sample capacity, which facilitates analysis of impurities at the 0.1% level or below. In the example of metoprolol this is exemplified in particular by the closely related *meta*-isomer compound 11. Also the synthesis impurities 2 and 12 have properties that in many systems make them prone to coelute with metoprolol. In packed column SFC these three related compounds are easily separated. The time for analysis by LC is about twice the time required by SFC.

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