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Supercritical fluid chromatography in the routine stability control of antipruritic preparations

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ABSTRACT

A recently developed system for supercritical fluid chromatography (SFC), based on independent flow and pressure control and suitable for packed and capillary columns, was tested on a routine level for the reliable, accurate and precise determination of active pharmaceutical substances in stability control. Only packed columns were used for this analysis. The chromatographic figures of merit and the validation data of the active substance alone and in two different dosage forms (accuracy, 98.8–99.2%; precision, 0.6%; linearity of response, 0.998–0.999) are comparable with the former liquid chromatographic methods. Economical (reduction of analysis time, fewer experimental steps and less sample pre-separation) and ecological (carbon dioxide instead of organic solvents) advantages make SFC an attractive alternative to liquid chromatography in the determination of crotamiton.

INTRODUCTION

In the last ten years liquid chromatography (LC) has become a very important analytical technique in pharmaceutical analytical laboratories [1], where it fulfills many of the needs for reliable, accurate and precise analysis [2]. Compounds of different polarities can be separated in one step by solvent programming if the absorption of the mobile phase does not cause a high baseline slope at low wavelengths (decreased sensitivity). However, an additional equilibration time is required to re-establish the initial conditions.

When the dosage forms are analysed, the LC column must be protected from the deposition of excipients by pre-separation of the active substance.

In contrast to liquids, supercritical fluids have physical properties which give a better mass transfer on the column [3]. As a consequence of this the column reaches equilibrium faster in supercritical fluid chromatography (SFC) than in LC after a mobile phase change [4]. Gradients (pressure, density, or modifier) can be used even at 210 nm without high baseline slopes [ultraviolet (UV) transparency of carbon dioxide, low modifier content]. As a result of the small amount of organic modifier used in the mobile phase, SFC can drastically reduce the consumption of organic solvents in the analysis.

The first-generation SFC instruments were based on syringe pump, capillary columns, flame ionization detection (FID) and pure carbon dioxide as the mobile

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phase [5]. However, the solvation power of carbon dioxide is not sufficient for the elution of very polar or ionic compounds, including many pharmaceutical preparations.

To extend the range of optimum separation conditions and chromatographable substances, special instrumental requirements must be fulfilled [gradients, pressure, density, or (and) modifier; columns, packed (PSFC) or open tubular capillary (CSFC); mobile phase, pure or on-line mixed] [6–10]. With such a second-generation instrument, SFC can be used on a routine basis even for highly polar compounds. This has not yet been shown in detail; the previous problems with polar compounds were cited as a major disadvantage of SFC [11].

On the basis of the author's experience, the advantages of SFC are discussed with respect to the quantitative analysis of different dosage forms of crotamiton, a compound used for the relief of itching.

EXPERIMENTAL

All experimental procedures and evaluation of data were conducted under the Good Laboratory Practice (GLP) guidelines.

Instrumentation

Fig. 1 shows a schematic diagram of a second-generation SFC system suitable for use with PSFC (A) or CSFC (B) [7–10].

A piston pump back-pressure regulator system [9] separates the mobile phase flow-rate and pressure regulation, which leads to the independent control of flow and pressure without any additional calculations [6,12–15].

The SFC system was based on commercial LC hardware (Gilson: two piston pumps 305 (1,2), manometric module 805 (3), mixer 811 (4), sample injector 231 (5)

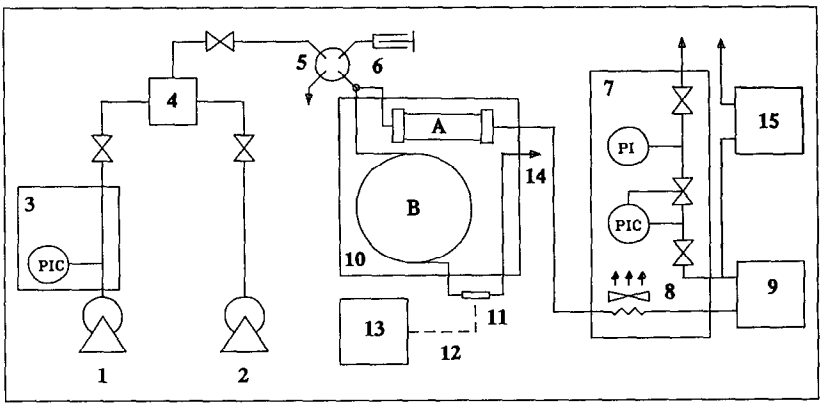

Fig. 1. Second-generation SFC system for PSFC (A) and CSFC (B) columns [7–10]. (1) Carbon dioxide pump, (2) modifier pump, (3) manometric module, (4) mixing chamber/dampener, (5) injection valve, (6) split for capillary column, (7) back-pressure control unit, (8) heat exchanger, (9) UV–visible detector, (10) column oven with PSFC (A) or CSFC (B) columns, (11) capillary UV–visible cell, (12) optical fibers, (13) UV–visible spectrophotometer, (14) heated restrictor–FID, (15) ELSD. (PI) Pressure indicator; (PIC) pressure indicator controller.

(valve Rheodyne 7413 with 1-μl internal loop or 7010 with 5-μl external loop) with diluter 401 combined with a laboratory-made back-pressure controle unit (7) described elsewhere [9]). A bourdon tube (coiled stainless-steel capillary, 2 m × 1/8 in. O.D., 1.8 mm I.D.) acted as an additional pulse dampener. It was placed between the mixer and the injection valve and was mounted in the unit containing the mixing chamber (4), where the temperature was >40°C [8].

A UV–visible absorption detector (9) (Spectra-Physics, Spectra-FOCUS) with an SFC high-pressure cell (volume, 250 nl; path length, 2 mm) was placed between heat exchanger (8) and pressure control unit (7). In certain cases a modified cell with a lower noise level at high flow-rates (volume, 1.6 μl; path length, 2 mm) was used.

An evaporative light scattering detector (ELSD) with a commercial SFC interface (s.e.d.e.r.e., SEDEX 45) was placed after the UV–visible detector to monitor the elution of the excipients in the crotamiton cream dosage form. In this instance the pressure control unit (7) was not used.

The LC instrumentation (Spectra-Physics, SP 8100) used for comparison was equipped with a 20-μl injection loop and UV–visible absorption detector (Spectra-Physics, Spectra-100).

A PS/2 computer (IBM) with a chromatographic software package (Spectra-Physics, LABNET) was used for data collection. Data evaluation was carried out with REPORT MANAGER and the SFC method validation with VAL (programs for Ciba-Geigy).

Columns

The chromatographic columns for SFC (Stagroma) were 100 and 250 × 2 mm, respectively, each equipped with a 20 × 2 mm pre-column and packed with 3-μm particles. Either Spherisorb CN or Nucleosil Si was used, depending on the application.

For LC a 125 × 4.6 mm column packed with Spherisorb Si 80 5-μm particles was used.

Although the dimensions and the particle size were different, the SFC and LC column types show sufficient and comparable resolution for the crotamiton analysis.

Chemicals

For SFC 99.90% grade carbon dioxide (Carbagas) was used as the primary mobile phase component and methanol (Fluka, HPLC grade) as a modifier. Trifluoroacetic acid and ammonium acetate (Merck, spectroscopy and p.a. grade, respectively) in methanol were used as binary modifiers for the chromatography of the crotamiton lotion dosage form.

Compound **1** (numbers refer to peaks in Figs. 2–4) is a possible by-product, 2-phenylethanol (**2**) and sorbic acid (**3**) are preservatives used in the lotion dosage form, **5** is a real by-product, *cis*-crotamiton (**4**) and *trans*-crotamiton (**6**) are together the active substance and **9** is a possible degradation product of the active substance.

The active substance crotamiton is also used in combination with hydrocortisone. Propylparaben and methylparaben (**7** and **8**) are preservatives contained in the cream dosage form, **10** is a possible by-product of hydrocortisone, **11** is a possible degradation product of hydrocortisone and **12** is the active substance hydrocortisone.

The placebo of all three dosage forms used contains the following excipients

without any active substance: (1) crotamiton cream preparation, Lanette O, glyceryl stearate, propylene glycol, liquid paraffin, isopropyl myristate, Myrj 52, water; (2) crotamiton lotion preparation, Lanette N, glyceryl stearate, propylene glycol, Cetomacrogol 1000, citric acid, Eutanol G, perfume creme 45, wool fat, water; (3) crotamiton–hydrocortisone cream preparation, stearyl alcohol, white petroleum, propylene glycol, perfume creme 45, water.

Solutions were prepared in tetrahydrofuran (THF) (Merck, chromatography grade) with the following concentrations of reference compounds: solution A (crotamiton), 2 ng/μl **1**, 1 μg/μl **4–6** and 5 ng/μl **9**; solution B (crotamiton 10% cream), same as solution A plus 10 μg/μl of the appropriate placebo cream; solution C (crotamiton 10% lotion), same as solution A plus 100 ng/μl, **2**, 7 ng/μl **3** and 10 μg/μl placebo lotion; solution D (crotamiton–hydrocortisone), 800 ng/μl **1**, 1.2 μg/μl **5**, 20 μg/μl **4–6**, 100 ng/μl **7** and **8**, 900 ng/μl **9**, 500 ng/μl **10**, 900 ng/μl **11**, and 10 μg/μl **12**. Only solution D must be cleared by filtration before injecting on to the SFC column.

For LC hexane and ethanol (Merck, HPLC grade) were used as the mobile phase. All LC reference solutions were prepared in the mobile phase with the following concentration of reference compounds: solution E (crotamiton), 0.2 ng/μl **1**, 119 ng/μl **4–6** and 0.5 ng/μl **9**.

RESULTS AND DISCUSSION

Method development

For the chromatographic method development the UVabsorption of the compound and its polarity affect the choice of detection mode (UV–visible spectrometry or evaporative light scattering [16,17], or FID), mobile phase polarity (carbon dioxide or carbon dioxide with modifier) and type and polarity of the column. Preference should be given to PSFC. Owing to the higher capacity, lower detection limits and shorter retention times. Compounds which cannot be eluted in their original form can sometimes be made suitable for SFC by derivatisation.

The most polar conditions for initial testing are: high modifier concentration (*e.g.* carbon dioxide + 25% methanol), high density (high pressure, 350–400 bar and low temperature, 40–60°C) and short, rather non-polar columns. Further selectivity can then be achieved through on-line (pressure density, modifier flow, temperature) and off-line (acids/bases in methanol, modifier change, longer and more polar columns) chromatographic variations.

Applications

The different polarities of the compounds present in the preparations mean that two to four chromatographic methods are required to detect each active substance and its corresponding by-products, degradation products and preservations. As a result of the greater analysis speed of SFC, the usefulness of SFC in comparison to conventional LC has been evaluated. PSFC with UV–visible detection is the method of choice.

Crotamiton

cis-Crotamiton (**4**) and *trans*-crotamiton (**6**) and their by-product (**5**) cannot be separated by reversed-phase LC, but can be separated with normal-phase LC as

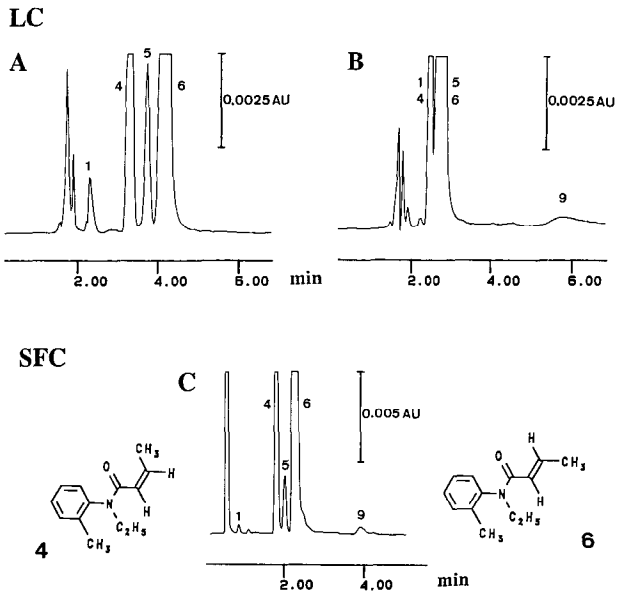

Fig. 2. Chromatograms obtained by (A and B) LC and (C) SFC of *cis*- (**4**) and *trans*-crotamiton (**6**). LC conditions (A and B): column, Si 80, 5 μm, 125 × 4.6 mm; mobile phase, hexane–ethanol (A = 94:6; B = 91:9); flow-rate, 0.9 ml/min; detection, UV absorption at 210 nm; sample, solution E. SFC conditions (C): column, CN. 3 μm, 270 × 2 mm; pressure, 125 (iso)bar; flow-rates, carbon dioxide 1.9 ml/min, methanol 0.05 ml/min; oven temperature, 100°C; detection, UV absorption at 210 nm; SFC cell volume, 1.6 μl; injection loop volume, 5 μl; sample solution A. For other compounds see under Experimental.

shown in Fig. 2A. The possible degradation compound (**9**) elutes very late and is too broad to be quantified. A second LC method using a higher-polarity mobile phase gives a better peak shape for this compound (**9**) as a result of faster elution (Fig. 2B). A combination of both isocratic methods by solvent programming is not usual in normal-phase LC.

With one simple SFC method all five of the important compounds for the analysis of crotamiton are separated under isobaric and isocratic conditions in less than 5 min (Fig. 2C) in the same elution order as in normal-phase LC. The chroma-

TABLE I

CHROMATOGRAPHIC FIGURES OF MERIT FROM THE *trans*-CROTAMITON RESPONSE PEAK AREA (NUMBER OF SAMPLES >20).

Parameter	LC	SFC
Response (% R.S.D.)	0.93	0.44
Retention time (% R.S.D.)	0.19	0.21
Tailing factor[a]	1.47	1.20
Resolution to neighbouring peaks	3.71	1.93
Proportionality of response	0.987	1.016

[a] Tailing factor, see Ref. 18.

TABLE II

LINEARITY, PRECISION AND ACCURACY OF THE SFC METHOD VALIDATION FOR CROTAMITON.

(A) Active substance; (B) active substance in cream; (C) active substance in lotion [19,20].

Statistic	A	B	C
Linearity	0.9998	0.9979	0.9998
Precision (% R.S.D.)	0.6	0.6	0.6
Accuracy (% recovery)	99.2	98.8	99.2

tographic figures of merit (Table I) for the LC and SFC analysis are comparable (tailing factor [18]). Data from the SFC method validation (Table II) are similar to the data from the LC validations.

For the method validation fifteen weighed samples with different concentrations of the active substance, alone or spiked into the appropriate placebo, were analysed (double injection). Linearity, precision and accuracy were calculated from the peak areas (Table II) [19,20].

The linearity of the detector response was measured with five samples of the active substance (50, 75, 100, 125 and 150%; the 100% value is given by the working procedure) and is expressed by the correlation coefficient r.

The precision is related to the variation of ten samples of the active substance alone or spiked into the appropriate placebo [75% (**2**), 100% (**6**) and 125% (**2**)] and is given as percentage relative standard deviation (RSD) based on the peak area relative to 1.0 mg.

The accuracy compares the weight of the sample with the weight found by the linearity curve. Six weighed samples of the active substance, either alone or spiked into the appropriate placebo [75% (**2**), 100% (**2**) or 125% (**2**)], were measured. The result is expressed as an averaged percentage recovery.

Crotamiton and hydrocortisone

A preparation containing crotamiton and hydrocortisone can also be analysed by a single SFC method using a modifier gradient (Fig. 3A). In addition to the five compounds of the crotamiton analysis, the preservatives (**7** and **8**) and the possible by-products and decomposition products (**10** and **11**) are separated from the active substance hydrocortisone (**12**). Additional pressure programming reduces the analysis time by about 0.5 min (Fig. 3B). Prior to the development of this method for stability testing of this preparation, four separate chromatographic methods were required.

Crotamiton dosage forms

To avoid precipitation on the column, a pre-separation of the active substance from the excipients (see under Experimental) is necessary when using the LC-methods.

For SFC the dosage forms (crotamiton cream and lotion) can be diluted in THF and injected directly into the SFC system without any pre-separation (Fig. 4A and C). The THF solution of the crotamiton–hydrocortisone cream must be cleared

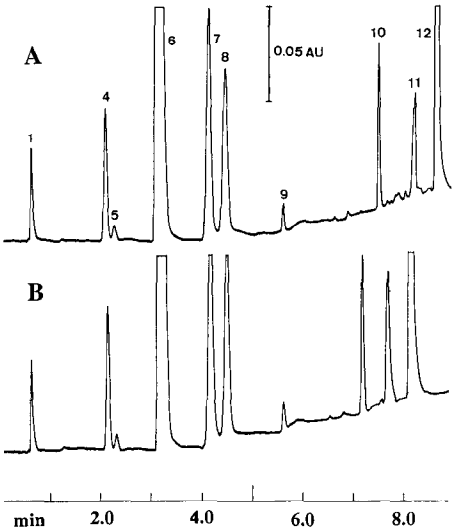

Fig. 3. SFC of crotamiton (**4** and **6**) and hydrocortisone (**12**) with (A) modifier gradient only or (B) modifier and pressure gradient. Conditions: column, Si and CN coupled, each 3 μm, 120 and 100 × 2 mm, respectively; pressure, 125 (iso)bar; flow-rates, carbon dioxide 2.0 ml/min, methanol 0.02 ml/min; oven temperature, 65°C; detection, UV absorption at 254 nm; SFC cell volume, 250 nl; injection loop, 1 μl. Gradients: (A) modifier, 3.5 min isocratic, 0.02 ml/min in 4 min linearly to 0.3 ml/min, 2.5 min isocratic, 0.3 ml/min; (B) modifier (see A) and pressure gradient, 4 min 125 (iso)bar, in 4 min linearly to 250 bar, 2 min 250 (iso)bar. For other compounds, see under Experimental.

by filtration. The elution of the excipients, which does not affect the quantitative UV absorption, is detected by a subsequent ELSD (Fig. 4B). This double detection gives information about the UV active and non-active compounds in just one chromatographic run.

The 10% crotamiton lotion (Fig. 4C) contains two additional preservatives (**2** and **3**) to that of the cream formulation (Fig. 4A). The use of trifluoroacetic acid as an additional binary modifier in the methanol modifier helps to elute the sorbic acid (**3**), but it prevents a secondary amine (**1**) from eluting. Mixing trifluoroacetic acid and ammonium acetate in the methanol modifier gives a satisfactory resolution for both compounds, the base (**1**) and acid (**3**), without any effect on the separation of the other important compounds.

The advantage of these two volatile additives, compared to others such as potassium heptanesulphonate, dimethyloctylamine and tributyamine [21], is their potential usefulness for SFC–ELSD and SFC–mass spectrometry (MS). SFC–MS is especially important for the identification of possible by-products and/or degradation products of active substances in pharmaceuticals.

In general, using SFC rather than LC as a method for the stability control of crotamiton results in both economical (time) and ecological (safety) advantages.

The economical advantages are the reduction of analysis time, fewer chromatographic methods and less sample pre-separation, which reduces the temporal expense of the former LC methods by a factor 3–4. The ecological advantages are the substitution of organic solvents with non-toxic and non-flammable carbon dioxide,

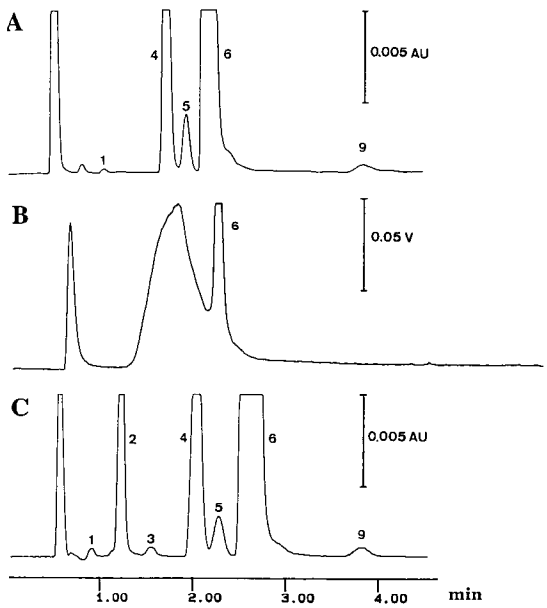

Fig. 4. SFC of crotamiton (**4** and **6**) in different dosage forms (A) cream (UV); (B) cream (ELSD); (C): lotion (UV). Conditions: column, CN, 3 μm, 270 × 2 mm; pressure, 125 (iso)bar; flow-rates, carbon dioxide 1.9 ml/min, methanol 0.05 ml/min; oven temperature, 100°C; detection, (A) UV 210 nm, SFC cell volume 1.6 μl, (B) ELSD 45°C; injection loop, 5 μl. (A and B) Modifier, neat methanol, sample solution B; (C) modifier, 0.035% trifluoroacetic acid and 0.035% ammonium acetate in methanol, sample solution C. For other UV active and non-active compounds see under Experimental.

which increases the laboratory safety and reduces environmental pollution (in this work, less than one tenth of the conventional amount of organic solvent is used).

As a result of these investigations SFC can be used as an alternative method for stability testing. It is suggested that a breakthrough in the use of SFC as a routine chromatographic technique in the laboratory and process control will be seen with the commercial production of second-generation instruments.

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