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Development of an orthogonal method for mometasone furoate impurity analysis using supercritical fluid chromatography

Zhenyu Wang*, Honggen Zhang, Oscar Liu, Brent Donovan

Pharmaceutical Science and Clinical Supply, Merck & Co., Inc., Summit, NJ 07901, USA

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ABSTRACT

While supercritical fluid chromatography (SFC) has received great popularity in chiral separation and purification, it has rarely been used for trace level pharmaceutical impurity analysis, partially due to the limitation of instrument sensitivity. In this study, a packed column SFC method has been developed for the quantitative analysis of mometasone furoate and its trace level impurities. The UV detection was optimized to improve the sensitivity by 2–4 fold. In combination with an increased sample concentration, this SFC method is capable of trace level (0.05% of the active) analysis of the impurities. The SFC method used a silica column and a mobile phase consisting of CO_2 and methanol. The new method provides an orthogonal selectivity complementary to the reversed phase HPLC (RP-HPLC) method. All of the impurities and the active were baseline separated within 12 min on SFC, which is less than one third of the RP-HPLC method run time. The method was also partially validated for linearity, accuracy, precision (repeatability), and limit of quantitation. This study demonstrated that the SFC method, with improved sensitivity, can be a valuable tool to provide orthogonal selectivity for trace level impurity separation. With further validation, the method may be suitable for release testing and stability testing for mometasone furoate drug substance.

1. Introduction

During the production of active pharmaceutical ingredients (API), it is highly likely that some impurities are generated as well. The safety of the drug not only depends on the toxicological properties of the active itself, but also on the impurities it contains. Therefore, it is critical that these impurities be quantified and/or identified, as prescribed by the ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) [1].

Practically, the analyst is dealing with relatively small quantities of process impurities or degradation products that often exhibit similar chemical structures to that of the active substance. In addition, and specifically during early phase development, the analyst often does not know the number of impurities or the structure of every impurity, which can make method development quite difficult [2]. For this reason, assessment of the impurity profiles of APIs is one of the most important and challenging activities in pharmaceutical analysis.

For the chromatographic methods used in pharmaceutical analysis, a common concern is that some impurity peaks may be overlooked due to coelution with another (possibly larger) peak in the chromatogram. Hence, the specificity is a critical attribute that should be thoroughly investigated and demonstrated in method development and validation [3]. Specificity, by definition, is the method's ability to unequivocally assess the individual analyte in the presence of other components. The specificity is especially important for the analytical method intended for early-phase drug development when the chemical and physical properties of the API are not fully understood and the synthetic processes are not fully in control.

Various approaches have been adopted to ensure method specificity. A feasible and reliable approach to check specificity is to develop a secondary method to separate peaks of interest using a different separation mechanism [4]. Ideally, the secondary method should be orthogonal to the primary method. Such a method should differ significantly in chromatographic selectivity by providing a completely different retention mechanism. It offers marked changes in relative retention so that two peaks which are unresolved in one method will likely be separated in the second method. In practice, to maximize the probability of revealing all peaks, the orthogonal method should be jointly developed with the primary method to provide adequate peak capacity and resolving power.

Reversed phase HPLC has been established as the main workhorse for impurity analysis during drug development and production. Typically, the secondary method has been pursued by varying the stationary phases (e.g. C18, C8, phenyl etc.) or the

* Corresponding author. Tel.: +1 908 473 4703; fax: +1 908 473 4629.
E-mail address: zhenyu.wang@merck.com (Z. Wang).

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mobile phases (acetonitrile, methanol, pH etc.) [4,5]. With this approach, a successful secondary method is capable of reversing the elution order of the critical pair or resolving co-eluted peaks. The risk of this approach is that the secondary method usually does not dramatically change the overall elution order for all the peaks so some peaks may still be overlooked. Other HPLC based separation techniques, such as hydrophilic interaction chromatography (HILIC) can provide more profound changes in retention. This method has been applied for impurity profiling [3].

Having a normal phase-like retention mechanism, packed column supercritical fluid chromatography (SFC) has the capability and potential to be utilized for orthogonal method development that is complementary to RP-HPLC. The most common mobile phases in SFC consist of a small amount of alcohol mixed with carbon dioxide (CO_2). The mobile phase is kept under supercritical or subcritical conditions via an electronically controlled variable pressure restrictor positioned after the detector. Carbon dioxide has favorable critical parameters, that is, a critical temperature of 31 °C and a critical pressure of 7.3 MPa. Supercritical CO_2 has unique physical properties: high diffusivity, low viscosity, and adjustable solvating power. Such distinct physical properties grant SFC some advantages over conventional HPLC, such as higher flow rates and shorter re-equilibration time [6]. Moreover, CO_2 is cheap, non-toxic and non-flammable. As CO_2 is the primary mobile phase, SFC greatly reduces the consumption of organic solvent, and therefore, is deemed as a green technology. For packed column SFC, it is generally preferred that compounds of interest have a solubility of 1 mg/mL or higher in an organic solvent (methanol, tetrahydrofuran etc.) [7].

SFC has seen an increasing amount of attention in the pharmaceutical industry over the past decade. It has been recognized as a very useful tool for chiral separation and chiral purification due to its advantages in speed, resolution, and cost savings over traditional HPLC. As the mass directed SFC becomes commercially available, more and more labs may begin using SFC for achiral purification as well [8]. The current commercial prep-SFC systems can provide variable capacity from small-scale (~mgs) to large-scale purification (~kgs). This allows analytical labs to support compound purification, enantiomer isolation, and impurity/degradant isolation quickly and effectively. Traditionally, the application of SFC has been thought to be limited to non-polar and moderately polar analytes because of the weak solvating power of the mobile phase. Recent studies have demonstrated that with the help of appropriate additives and stationary phase, it is feasible to use SFC to separate highly polar compounds such as pharmaceutical salts, nucleobases, and even polypeptides [9–11].

Despite its increasing popularity in the pharmaceutical industry, most implementations of SFC are in drug discovery, where the applications are generally qualitative and semi-quantitative. SFC instruments are much less common in drug development labs where the analytical activities and requirements are quite different. General analytical activities in drug development include assay testing (potency, content uniformity, and dissolution), and impurity testing. Among these tests, the impurity testing of the drug substance or drug product is currently the most important analysis because it is directly related to drug safety and shelf life. Impurity testing is also one of the most challenging and time-consuming tasks, as it requires an analytical method having adequate selectivity and sensitivity to separate and quantify a complex mixture of structurally related analytes at trace level.

A review of the SFC literature revealed that very few papers reported using SFC for pharmaceutical impurity analysis [12,13]. One of the major obstacles is that the existing SFC-UV instruments are less sensitive compared to HPLC-UV instruments. This makes the detection of trace level impurity very challenging. In this study, a simple approach has been explored to improve SFC sensitivity. Mometasone furoate (Fig. 1) with spiked known impurities was used as the probe. A method with orthogonal selectivity to RP-HPLC was achieved on SFC. The study has demonstrated that SFC with improved sensitivity can be used as a complementary tool to RP-HPLC for pharmaceutical impurity profiling, separation, and quantitation.

2. Materials and methods

2.1. Instrumentation

The SFC study was conducted using a TharSFC Method Station Analytical System (TharSFC, Pittsburgh, PA) equipped with a fluid delivery module, column oven, autosampler, automated back pressure regulator, and Waters 2998 photodiode array (PDA) detector. The injection loop was 20 μL in volume. Instrument control and data collection were conducted using Waters Empower 2 software.

RP-HPLC analysis was performed on an Alliance 2690 HPLC system equipped with a 2996 PDA detector and a column heater (Waters, Milford, MA). The injection volume was 20 μL. The LC method was modified from the literature and compendial methods [14–16]. Mobile phase A was de-ionized water and mobile phase B was acetonitrile. A gradient method (42% B to 52% B in 60 min) was used for reversed phase separation of mometasone furoate and its impurities. Flow rate was 1.5 mL/min.

The following columns were used during SFC method development: silica column (250 mm × 4.6 mm, 5 μm particle size) (Kromasil, Bohus, Sweden), 2-ethylpyridine column (250 mm × 4.6 mm, 5 μm particle size) (Princeton Chromatography, Princeton, NJ), and cyano column (250 mm × 4.6 mm, 5 μm particle size) (Restek, Bellefonte, PA). An Ultrasphere ODS column (250 mm × 4.6 mm, 5 μm particle size) (Beckman, Brea, CA) was used for the reversed phase HPLC separation.

2.2. Materials

Carbon dioxide (SFC grade) was purchased from Airgas (Radnor, PA). HPLC grade methanol, ethanol, and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ). In-house de-ionized water was filtered through a Millipore system using a 0.22 μm filter (Bedford, MA). Reference standards for mometasone furoate and its eight isolated related impurities (Merck Research Laboratories) were used in this study. The structures of compounds 1, 3, 5, 7 and 8 are shown Fig. 1. Others are proprietary compounds of Merck & Co. (their structures are not presented in this report). Methanol was used as the sample solvent for mometasone furoate and its impurities during SFC analysis.

3. Results and discussion

Mometasone furoate is a very potent glucocorticoid anti-inflammatory agent and the active ingredient of several pharmaceuticals including Elocon® cream/ointment, Nasonex® nasal spray, Asmanex Twisthaler®, and Dulera® inhalation aerosol. Eight isolated process impurities and degradation products were separately prepared then spiked with mometasone furoate (API). This mixture was used during SFC method development. Peaks were tracked based on the retention time of individual impurities injected.

3.1. SFC method development

Similar to the method development for HPLC, the selectivity tuning on SFC can be divided into two parts; “coarse” adjustment by changing the stationary phase, and “fine” tuning by changing

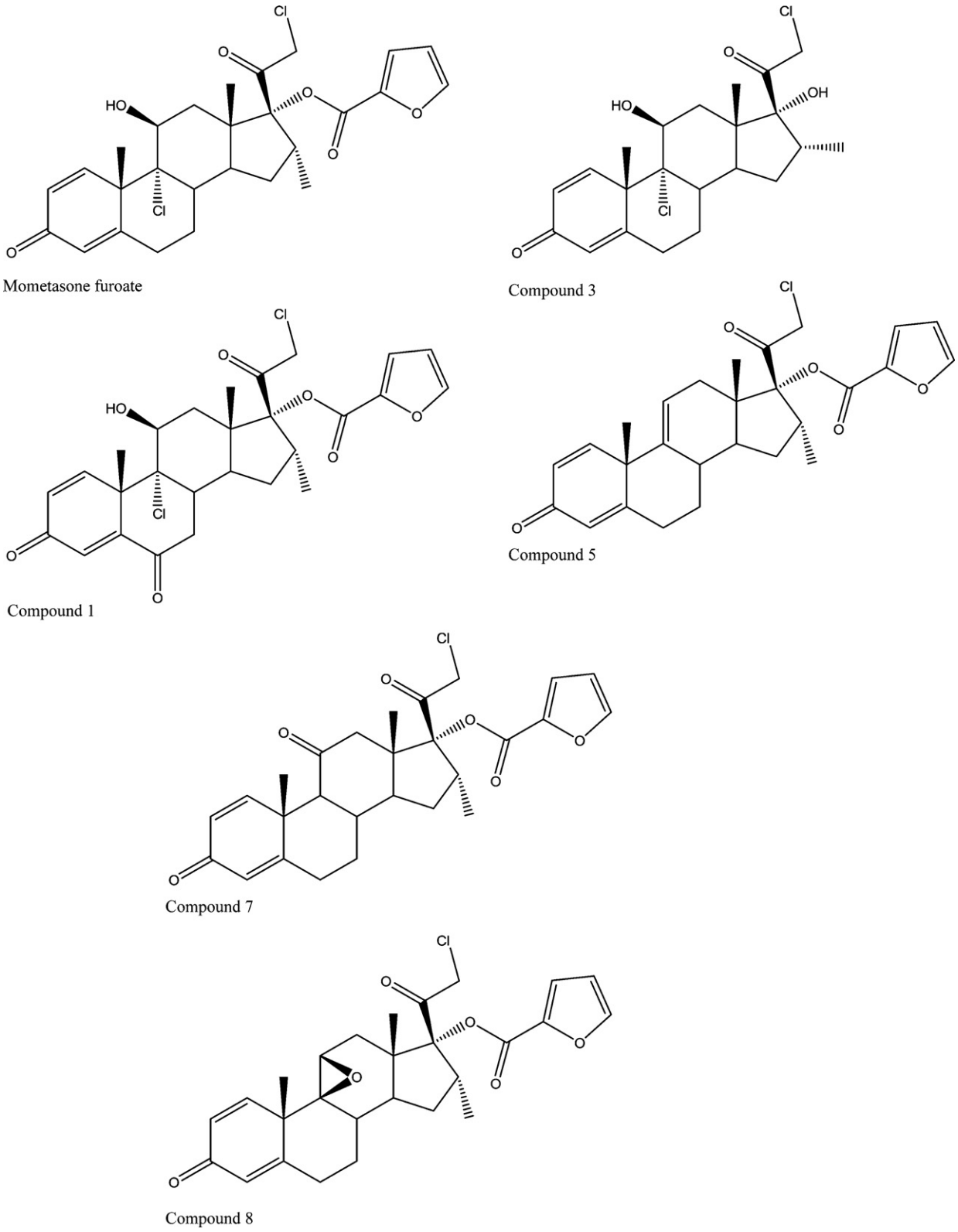

Fig. 1. Chemical structures of mometasone furoate and compounds 1, 3, 5, 7 and 8.

the mobile phase or other experimental parameters. For the initial screening, CO_2/methanol can be utilized as the mobile phase. For acidic or basic compounds, some additives (acid and base, respectively) may be considered to improve peak shape and facilitate elution. Multiple normal phase columns (e.g. silica, cyano etc.) should be screened with gradient elution. The goal of column screening was to find a suitable stationary phase that provides maximum selectivity towards all individual analytes. Three normal phase columns, namely 2-ethylpyridine, cyano, and silica were evaluated. Both cyano and silica columns have traditionally been used on normal phase LC. The 2-ethylpyridine column was developed more specifically for SFC applications and has achieved wide

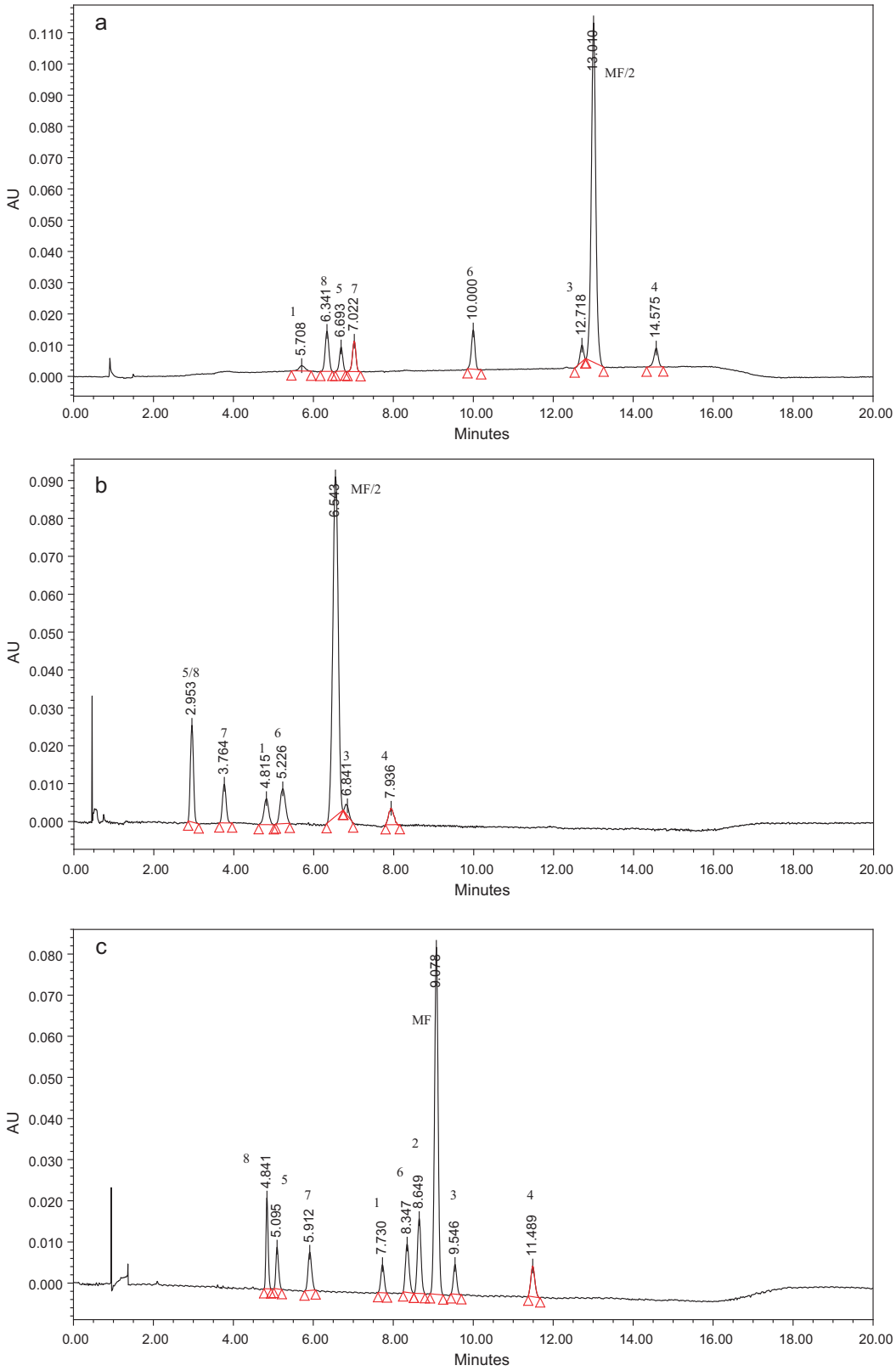

Fig. 2. Column screening on: (a) 2-ethylpyridine, (b) cyano, and (c) silica columns. CO_2 100 bar, 4.0 mL/min, 30 °C, modifier: methanol 5–15% in 15 min.

success in the separation of various pharmaceutical compounds [17]. For the initial screening, neat methanol was used as the modifier. Considering the analytes are neutral compounds, no additive was used. A shallow gradient (5–15% methanol) was used to screen these columns to separate the spiked mixture. As shown in Fig. 2a, on the 2-ethylpyridine column, compound 2 co-eluted with mometasone furoate. Compound 3 was only partially separated from the main peak. On the cyano column (Fig. 2b), compound 5 and compound 8 were co-eluted, and compound 2 co-eluted with the main peak. Compound 3 eluted at the tail of the main peak

with partial resolution. The best separation was achieved on the silica column (Fig. 2c) with all nine components baseline separated within 12 min.

Further fine tuning focused on the silica column and the evaluations of several parameters including modifier, pressure and temperature. It is well established that the selectivity and efficiency on packed column SFC are influenced by the nature of the organic modifier [6]. Other than methanol, both ethanol and acetonitrile were compared as potential modifiers in this study. When moving from methanol to a less polar alcohol, in this case ethanol, an increase in the retention for all the components as well as several co-eluting peaks was observed. When acetonitrile was used as the modifier, more peaks co-eluted with mometasone furoate. Therefore, it was determined that methanol be used as the modifier in this method.

In SFC, the solvation strength of the mobile phase is governed primarily by the nature of the modifier, but it is also determined in some degree by the density of the mobile phase. In SFC, changing the density of the mobile phase by adjusting the pressure can be employed as an additional tool for selectivity tuning. Therefore, system back pressure at 100 bar and 150 bar were both evaluated (Fig. 3). As the pressure is increased, both the density and solvation strength of the mobile phase are increased. This explained the decreased retention at higher system pressure, as shown in Fig. 3b. However, the resolution was compromised at higher system pressure and compound 1 was only partially separated from compound 6. As the pressure increased, a significant increase in baseline noise was also observed. In packed column SFC instruments, the compressed mobile phase is maintained under pressure via an automatic back pressure regulator (BPR), which is located downstream of the UV detector. Based on the feedback of the pressure sensor, the electronic BPR needs to constantly adjust to maintain the desired pressure. For the type of BPR that is being used in this study, system pressure is maintained by adjusting the distance between the needle and the needle-seat to change the size of the orifice. The frequent movement of the BPR produces subtle pressure pulses which are pronounced enough to affect the adjacent UV detector [18]. The higher pressure setting apparently increases the workload of the BPR and results in a noisier baseline.

Changing the temperature (at constant pressure) will change the density of the SFC mobile phase and the kinetic energy of the solute. The combination of these effects can be used for retention/selectivity adjustment. Temperatures of 30 °C, 35 °C, and 40 °C were evaluated while the pressure was kept constant at 100 bar to reduce the baseline noise. The column temperature control component can provide robust temperature control at ~5 °C above the room temperature. For this reason, the lowest temperature evaluated was 30 °C. As shown in Fig. 3c, when column temperature was increased from 30 °C to 35 °C, the overall retention time increased. This may be due to reduced density of the mobile phase at higher temperature (when pressure remained constant). There is also a slight decrease in selectivity. Compound 2 and compound 6 were partially separated at 35 °C. When temperature was further increased to 40 °C (Fig. 3d), the trend of increased retention and deteriorated selectivity became more pronounced. Compound 3 and mometasone furoate co-eluted at elevated temperature as did compound 6 and compound 2. For the remaining experiments, the column temperature was maintained at 30 °C.

SFC-UV is seldom used for trace level analysis. One of the major concerns for existing SFC instrumentation design is inadequate sensitivity. Packed column SFC has "downstream" pressure control where outlet pressure is dynamically controlled using the BPR. Pressurized mobile phase naturally tends to depressurize back to its stable state (i.e. gaseous) via an endothermic process. Due to this characteristic, UV detection is more sensitive to thermal and mechanical changes with SFC than with HPLC. Any subtle thermal and mechanical fluctuations may increase the noise level on a SFC-UV trace [18]. One simple approach to improve the sensitivity on the existing SFC instrument is to use the Reference Wavelength Compensation function [19]. This function is a common built-in feature of photodiode array (PDA) detectors. The compensated reference wavelength collects wide-band absorbance data in a region of the spectra where no analytes are expected to appear. The detector calculates the compensation value by averaging the absorbance values within the selected range of wavelengths. This averaged value is then subtracted from the absorbance value to obtain the compensated absorbance. This approach can reduce the non-wavelength-dependent noise such as detector drift and wander, and thereby increase the overall sensitivity.

During the UV sensitivity optimization study, the absorbance wavelength was chosen at 245 nm where mometasone furoate has the maximum UV absorbance. The compensation wavelength was chosen at 400–440 nm where the analytes have no absorption. To take full advantage of the wavelength compensation feature, several detector settings were evaluated and optimized, namely resolution, filter constant, and sampling rate. The results of the UV detection sensitivity study are summarized in Table 1. In general, two to four fold increases in sensitivity were observed when the wavelength compensation function was used. For each experiment, there were adequate data points (>25) across the peak. In this study, the peak width values ranged from 0.066 to 0.109, depending on the UV detection parameter settings. Among all the combinations of detector settings, three settings were chosen for further evaluation using the mometasone furoate impurity mixtures and the chromatograms were compared. These three settings each gave good signal-to-noise ratio and different peak widths (narrow, median, and broad). The setting "vii" gave the highest signal to noise ratio due to the smoothest baseline. However, the trade-off was loss of resolution due to severe band broadening. As a result, compound 2 and compound 6 were only partially separated. The setting "iii" gave a slightly higher value on peak width than the setting "ii". There was virtually no difference between the chromatograms collected using these two settings. The setting "iii" was chosen as the final detection parameter because of its higher sensitivity (than the setting "ii").

The final optimized separation conditions are: silica column, CO_2 100 bar, 30 °C; 5–15% methanol in 15 min and total flow rate of 4 mL/min. Detection at 245 nm with wavelength compensation from 400 to 440 nm, sampling rate 5, resolution 3.6 nm, filter constant slow. At this optimized condition, mometasone furoate and its impurities are baseline separated and the final peak is eluted within 12 min.

3.2. SFC method validation

The optimized method was further validated for linearity and range, accuracy (assay and impurity levels respectively), precision (repeatability), and limit of quantitation. As a common practice in pharmaceutical analytical labs, it is preferred that the assay (potency) test and impurity test be performed as one test to reduce the workload. In other words, one analytical method should be capable of handling both the assay and impurity tests. For this reason, the nominal concentration (assay level) of this method was chosen at 2.0 mg/mL so that a desired relative sensitivity (0.1% of the nominal) could be achievable. In the validation experiment, the method was deliberately validated at both the impurity and assay levels.

The linearity of the method was determined by analyzing five serially diluted concentration levels ranging from 0.1% to 120.0% of the nominal concentration. Regression analysis of the peak area versus concentration data yielded a coefficient of determination (R^2) > 0.9999 for mometasone furoate (Table 2).

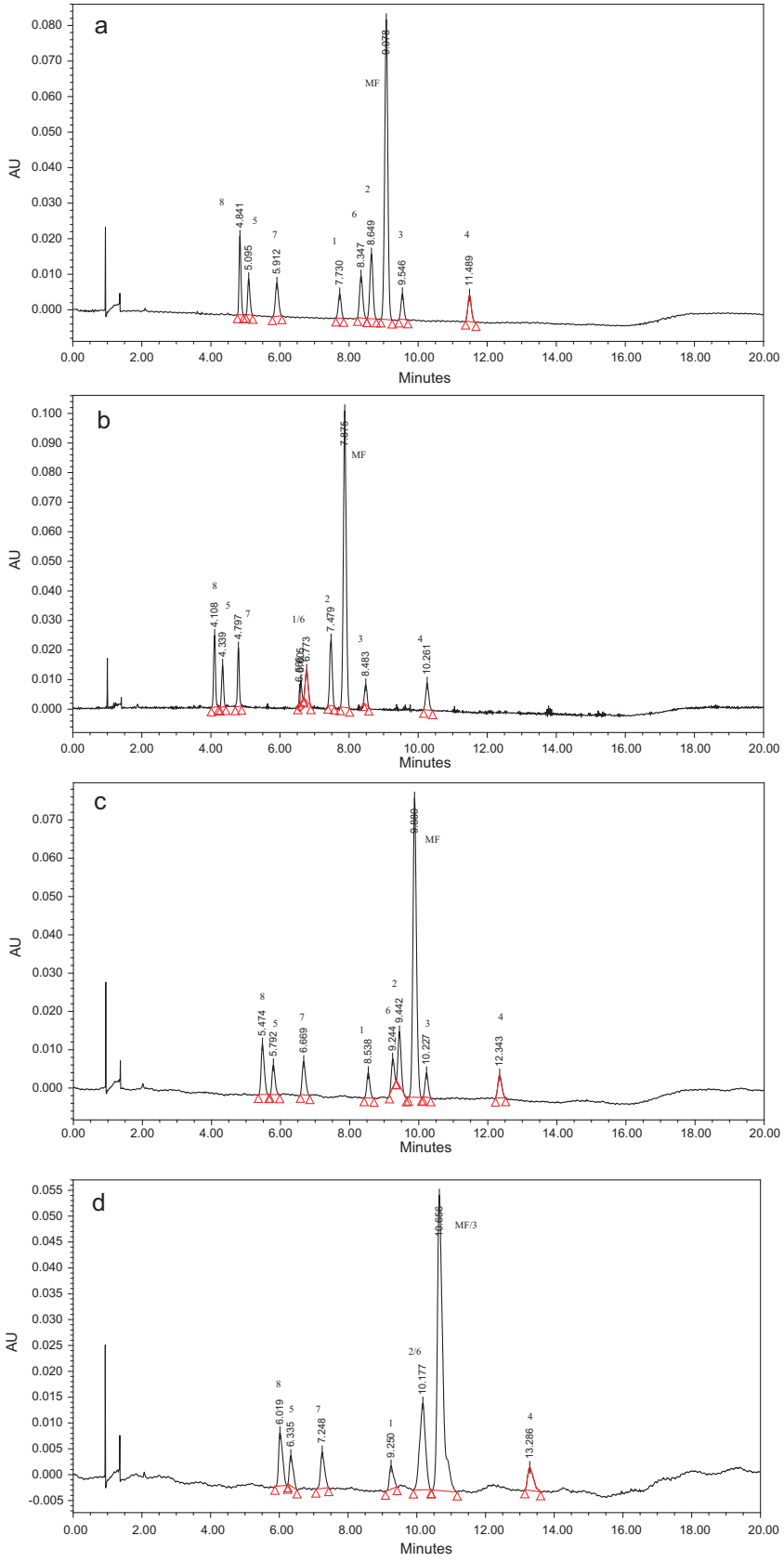

Fig. 3. Evaluate mobile phase pressure and column temperature impact on selectivity. (a) 100 bar, 30 °C; (b) 150 bar, 30 °C; (c) 100 bar, 35 °C; (d) 100 bar, 40 °C. 4.0 mL/min, modifier: methanol 5–15% in 15 min, silica column.

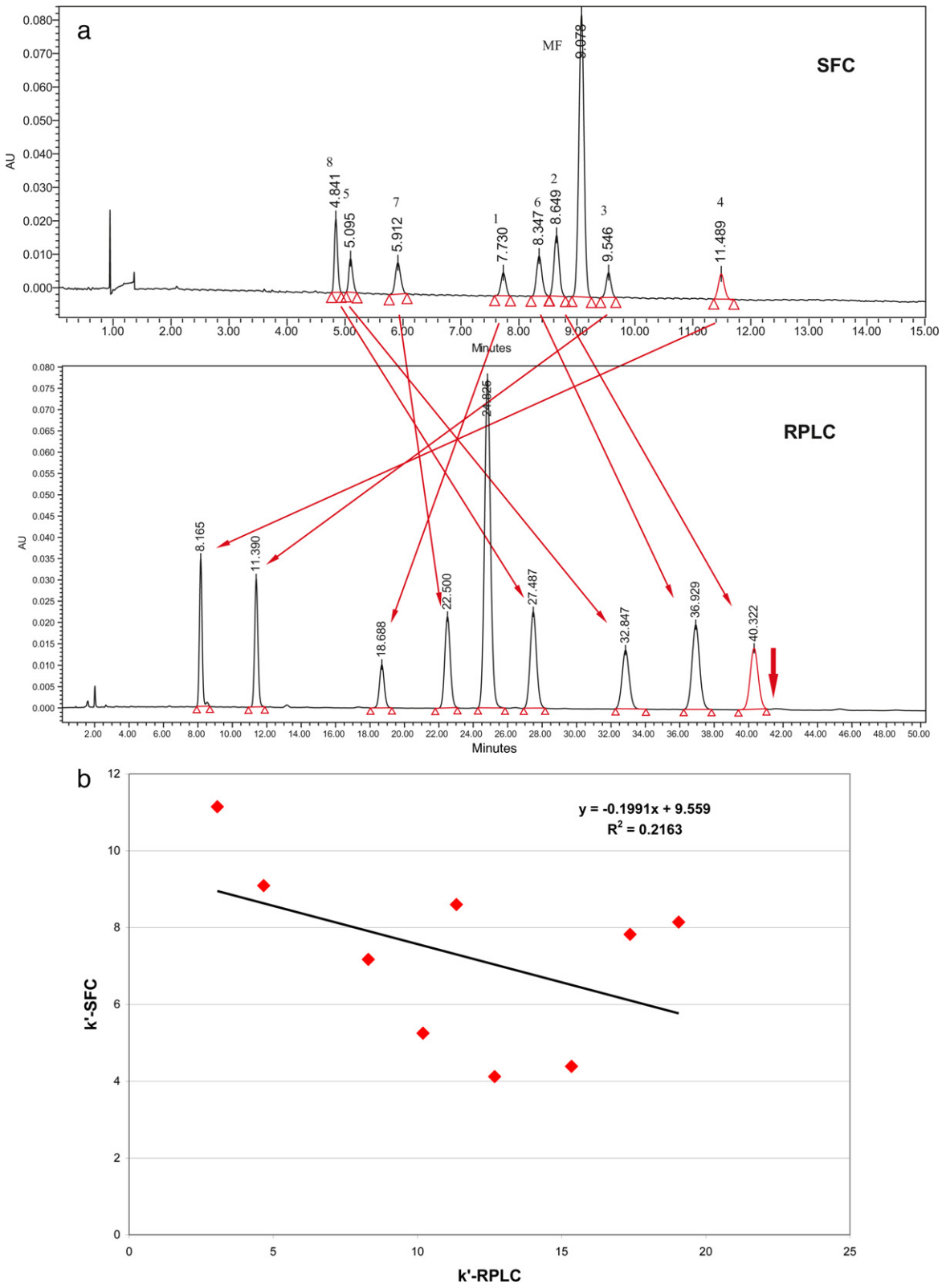

Fig. 4. (a) The orthogonal selectivity of SFC method vs. RP-HPLC method. SFC condition: 100 bar, 30 °C, 4.0 mL/min, modifier: methanol 5–15% in 15 min, silica column. RP-HPLC condition: 25 °C, 1.5 mL/min, water/acetonitrile (58:42, v/v) to water/acetonitrile (48:52, v/v) in 60 min, Ultrasphere ODS column. (b) Comparison of retention factor (k') in SFC method and in RP-HPLC method.

The accuracy of the method was determined at both assay level and impurity level. The accuracy at assay level was evaluated with six preparations of mometasone furoate at the nominal concentration level (2.0 mg/mL) and the recovery was between 99.8% and 101.6%. The accuracy at impurity level was determined with six preparations of individual spiked impurities at 0.1% of the nominal concentration (final concentration of 0.002 mg/mL). The average recovery ($n = 6$) of spiked individual impurities was between 88.3%

Table 1
Summary of sensitivity improvement study.

PDA detector settings				Without wavelength compensation		With wavelength compensation		Improvement in sensitivity
	Sampling rate	Bandwidth	Filter constant	Peak width	S/N[a]	Peak width	S/N[b]	$(S/N^b)/(S/N^a)$
i	5	2.4	Slow	0.072	67	0.072	151	2.2
ii[c]	5	2.4	Normal	0.066	44	0.066	139	3.2
iii[c]	5	3.6	Slow	0.073	57	0.072	163	2.9
iv	5	3.6	Normal	0.066	62	0.066	122	2
v	5	4.8	Slow	0.072	62	0.072	142	2.3
vi	5	4.8	Normal	0.066	54	0.067	115	2.1
vii[c]	2	2.4	Slow	0.108	62	0.108	251	4
viii	2	2.4	Normal	0.077	71	0.077	170	2.4
ix	2	3.6	Slow	0.108	52	0.108	190	3.7
x	2	3.6	Normal	0.077	47	0.077	159	3.4
xi	2	4.8	Slow	0.109	63	0.108	175	2.8
xii	2	4.8	Normal	0.077	43	0.077	162	3.8

[a] Duplicate injections (~0.001 mg/mL of mometasone furoate solution) per experiment and average value were reported.
[b] Peak width was measured at the half of the peak height.
[c] Parameters were further evaluated using impurity mixtures.

and 104.7%. Good precision was obtained as determined by the relative standard deviation (RSD). RSD was 0.7% ($n = 6$) at the assay level. The recovery precision of each impurity was in the range of 1.4–5.4%. The limit of quantitation of the method was determined to be 0.05% (relative to the active) for the impurities at a signal to noise ratio of 10.

3.3. Comparison with RP-HPLC method

With the goal of providing an orthogonal selectivity to the RP-HPLC method, we compared the elution order of these two separation techniques in Fig. 4a (HPLC vs. SFC). Due to a mechanism similar to normal phase HPLC, SFC provides a dramatically different retention order compared to that of RP-HPLC. The orthogonality of two chromatographic separations is also quantitatively described by calculating the correlation coefficients of retention factors from two methods. The coefficient of determination (R^2) is only about 0.2, which indicates a significant selectivity difference between two separation methods (Fig. 4b).

The results from the above SFC method validation were then compared to that of the RP-HPLC method (Table 2). Identical injection volume (20 μL) and same type of detector were used in the LC method. The LC separation was achieved using an ODS column which has the same dimension as that of the silica column (250 mm × 4.6 mm, 5 μm) used on SFC. Both methods have good linearity in a wide range of sample concentrations. The accuracy and precision are highly comparable between the two methods at both assay and impurity levels.

Table 2
Comparison of RP-HPLC and SFC method validation results.

	RP-HPLC method	SFC method
Sample concentration	0.2 mg/mL	2.0 mg/mL
Linearity	0.9999	0.9999
Accuracy		
Assay level[a]	99.1–100.7%	99.8–101.6%
Impurity level[b]	96.6–115.4%[c]	88.3–104.7%[c]
Precision		
Assay level[a]	0.4%	0.7%
Impurity level[b]	1.9%–5.0%	1.4%–5.4%
Limit of Quantitation	0.05% (or 0.1 μg/mL)	0.05% (or 1.0 μg/mL)

[a] Six preparations ($n = 6$).
[b] Six preparations of spiked individual impurities.
[c] The average recovery of each impurity was reported.

However, the SFC method is about 10 times less sensitive than that of RP-HPLC method, even after the detection sensitivity optimization. To overcome this drawback, as described in Section 3.2, the sample solution concentration was chosen to be 2.0 mg/mL so that the SFC method can achieve the relative sensitivity for the low level impurities (QL = 0.05% of the active). This approach is unlikely achievable for a RP-HPLC method, but luckily manageable for SFC. It is well known that the sample solvent in the RP-HPLC method is preferred to match the composition of the starting gradient. If the organic portion in the sample solvent is significantly higher than that in the starting solvent of gradient (or isocratic) mobile phase, the peak shape of analytes may be distorted. A certain percentage of water is therefore required in the sample solvent, which may reduce the analyte solubility in the sample solvent. Luckily for those compounds with high solubility in organic solvents, high sample concentration or compound solubility is not a challenge in analytical SFC since neat organic is typically used as the sample solvent. To compensate for the sensitivity limitation, using a higher sample concentration could be a viable solution as long as the method meets the validation and performance criteria (e.g. carry over).

4. Conclusion

Compared to well-established technology such as reversed phase LC, packed column SFC may provide higher separation efficiency and faster analyses with less consumption of organic solvent. SFC also offers chromatographic separation selectivity that is often similar to that of normal phase LC. This is an attractive feature that complements commonly used reversed phase LC systems. Despite these advantages, SFC has rarely been used in pharmaceutical impurity analysis partially due to its inadequate sensitivity. In this study, the wavelength compensation function was utilized and optimized to reduce the noise level and improve the UV sensitivity (about 2–4 fold). A truly orthogonal chromatographic method was developed on SFC for the analysis of mometasone furoate and its impurities. The selectivity is complementary to that obtained on the RP-HPLC method. The SFC separation parameters were optimized to maximize the resolution and sensitivity. The SFC method provides baseline separation for all components with an analysis time less than one third of that required by the LC method. The SFC method has been partially validated and demonstrated good linearity, accuracy, and precision at both the assay and impurity levels.

Although this SFC method is about 10 times less sensitive than the RP-HPLC method which uses the same type of PDA detector and the same dimension of column, the unique separation mode of SFC allows for the use of neat organic as the sample solvent to obtain higher sample concentration. This approach may overcome SFC's limitations in sensitivity and allow for trace level analysis (0.05% of the active). Nevertheless, a significant improvement in the SFC instrumentation design is desired to fundamentally improve the instrument sensitivity. This improvement could dramatically expand the utility of SFC in more regulated environments and to a much wider range of applications.

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References

[1] International Conference on Harmonization of Technical Requirements for 453 Registration of Pharmaceuticals for Human Use: Impurities in New Drug 454 Substances Q3A.(R2).
[2] E. Van Gyseghem, S. Van Hemelryck, M. Daszykowski, F. Questier, D.L. Massart, Y. Vander Heyden, J. Chromatogr. A 988 (2003) 77.
[3] X. Wang, W. Li, H.T. Rasmussen, J. Chromatogr. A. 1083 (2005) 58.
[4] J. Pellett, P. Lukulay, Y. Mao, W. Bowen, R. Reed, M. Ma, R.C. Munger, J.W. Dolan, L. Wrisley, K. Medwid, N.P. Toltl, C.C. Chan, M. Skibic, K. Biswas, K.A. Wells, L.R. Snyder, J. Chromatogr. A. 1101 (2006) 122.
[5] E. Van Gyseghem, I. Crosiers, S. Gourvenec, D.L. Massart, Y. Vander Heyden, J. Chromatogr. A. 1026 (2004) 117.
[6] T. Berger, Packed Column Supercritical Fluid Chromatography, Cambridge, UK, 1995.
[7] K. Anton, S. Siffrin, Analusis 27 (1999) 691.
[8] J. Van Anda, Am. Pharm. Rev. 13 (2010) 111.
[9] J. Zheng, L.T. Taylor, J.D. Pinkston, Chromatographia 63 (2006) 267.
[10] M. Ashraf-Khorassani, L.T. Taylor, J. Sep. Sci. 33 (2010) 1682.
[11] J. Zheng, J.D. Pinkston, P.H. Zoutendam, L.T. Taylor, Anal. Chem. 78 (2006) 1535.
[12] N.K. Jagota, J.B. Nair, R. Frazer, M. Klee, M.Z. Wang, J. Chromatogr. A 721 (1996) 315.
[13] J.L. Bernal, M.J. del Nozal, H. Velasco, L. Toribio, J. Liq. Chromatogr. Related Technol. 19 (1996) 1579.
[14] X. Teng, K. Foe, K. Brown, D. Cutler, N. Davies, J. Pharm. Biomed. Anal. 26 (2001) 313.
[15] European Pharmacopoeia (Version 6): Mometasone Furoate monograph.
[16] United States Pharmacopoeia (Version 33): Mometasone Furoate monograph.
[17] C. Brunelli, Y. Zhao, M. Hanna-Brown, P. Sandra, J. Sep. Sci. 31 (2008) 1299.
[18] Z. Wang, O. Liu, B. Donovan, Am. Pharm. Rev. 12 (2009) 94.
[19] L. Subbarao, J. Cole, R. Chen, LC–GC Application Notebook, September 2009.