Sensitive determination of impurities in achiral pharmaceuticals by supercritical fluid chromatography using the Agilent 1260 Infinity Analytical SFC System

Application Note

Pharmaceuticals

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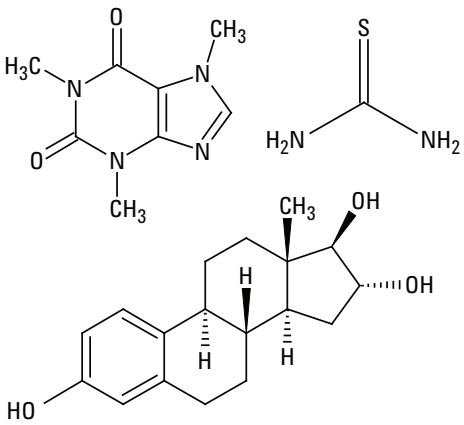

Abstract

Supercritical Fluid Chromatography is an alternative and complementary method to HPLC for the determination of impurities in pharmaceutical products. This study evaluated the performance of the Agilent 1260 Infinity Analytical SFC system for the analysis of thiourea in two model compounds. The study included both isocratic and modifier gradient modes. The resulting data showed excellent linearity and repeatability. Retention time repeatability was better than 0.1%. Limits of detection for the impurity determination were below 0.01% w/w relative to the main compound, and the RSDs of peak area were below 1% at the 0.5% concentration level and below 5% at the 0.05% concentration level in all cases. These values are well within acceptable method validation ranges.

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Introduction

The potential of Supercritical Fluid Chromatography (SFC) using packed columns for the analysis of impurities in pharmaceutical compounds has been recognized for many years. SFC can offer highly efficient separations in short analysis times and at a low pressure drop. However, the lack of reliable and sensitive commercial SFC systems has prevented the extensive use of SFC in the pharmaceutical industry. One major drawback is the higher background of supercritical carbon dioxide in UV/DAD detection in comparison to water, methanol or acetonitrile, resulting in lower sensitivity compared to state-of-the-art HPLC. Precise control of CO_2 flow, modifier mixing and back pressure are important to minimize noise and drift. These are essential instrument requirements for detecting low level impurities at 0.05-0.1% levels, relative to the active pharmaceutical ingredient (API).

The Agilent 1260 Infinity Analytical SFC System was tested for the analysis of thiourea, which is a model impurity with relatively low UV absorbance often used as a test solute to determine method performance (sensitivity). Caffeine and estriol were used as model API matrices, with polar and apolar characteristics, respectively. Under the SFC conditions used, thiourea elutes before estriol and after caffeine. The test included both isocratic and gradient analyses using a generic SFC method, and evaluated sensitivity, reproducibility and linearity.

Experimental

Chemicals

Stock solutions of thiourea, caffeine and estriol were prepared in methanol at approximately 10 mg/mL. These stock solutions were mixed in appropriate concentrations to obtain test solutions containing 5 mg/mL of main compound (caffeine or estriol) and, respectively, 0.5 µg/mL, 2.5 µg/mL, 5 µg/mL and 25 µg/mL of thiourea. These concentrations of thiourea correspond to respectively 0.01%, 0.05%, 0.10% and 0.50% relative to the model compound.

The peak identification name, chemical names, and structures of the compounds are listed in Table 1.

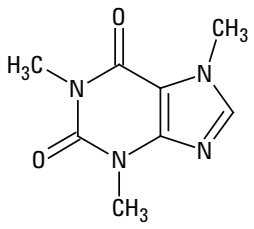
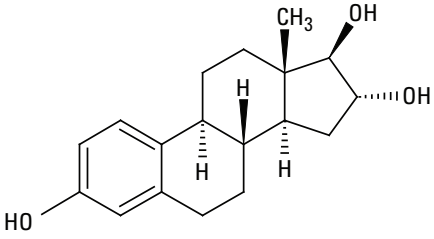
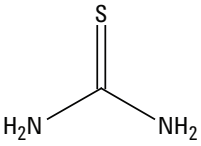
Peak	Name	Structure
Main	Caffeine	
Main	Estriol	
Impurity (X)	Thiourea	

Table 1
Test compounds.

Instrumentation

An Agilent 1260 Infinity Analytical SFC system (G4309A), consisting of an Aurora SFC Fusion A5 and a modified Agilent RRLC binary system was used.

All analyses used a normal phase column (Agilent ZORBAZ RX-Sil, 25 cm x 4.6 mm id, packed with 5 µm particles) as well as the same modifier (20 mM ammonium formate in methanol). Thiourea was detected at 254 nm in the caffeine matrix and at 220 nm in the estriol matrix. The experimental conditions are summarized in Table 2.

Instrument Conditions	
Column	Agilent ZORBAX RX-SIL, 4.6 mm × 250 mm, 5 µm
Supercritical Fluid	CO_2
Modifier	MeOH w 20 mM NH_4COOH
Outlet Pressure	150 bar
Flow Rate	2.0 mL/min
Isocratic Gradient A (caffeine) Gradient B (estriol)	25% modifier 0-6 min: 20 to 50% modifier 0-6 min: 20 to 30% modifier
Temperature	40 °C
Injection Volume	5 µL
Detection	DAD, 254 nm for caffeine DAD, 220 nm for estriol

Table 2
Experimental conditions.

Results and Discussion

Isocratic Separation

The study developed an isocratic method for the separation of the impurity from each of the main components. The chromatograms for the spiked samples at the 0.05% w/w level are shown in Figures 1A and 1B for caffeine and estriol, respectively. Thiourea can easily be detected in both matrixes at 3.1 min.

Figure 1B also illustrates some additional low level impurities in the estriol sample in addition to the spiked thiourea. These impurities are present at very low concentrations (< 0.01%, assuming similar response compared to thiourea).

This example illustrates the high sensitivity possible with the Agilent 1260 Infinity Analytical SFC System. The detector noise measured at 4.5–5.0 min in the caffeine sample and at 3.3–3.6 min in the estriol sample. Baseline noise (six times the SD) was below 0.1 mAU at 254 nm and below 0.2 mAU at 220 nm.

Table 3 summarizes the validated isocratic methods and the results. A series of samples spiked at the 0.01%, 0.05%, 0.1% and 0.5% (w/w) levels were analyzed. Linearity, expressed as r^2, was 0.9988 for thiourea in caffeine (254 nm)

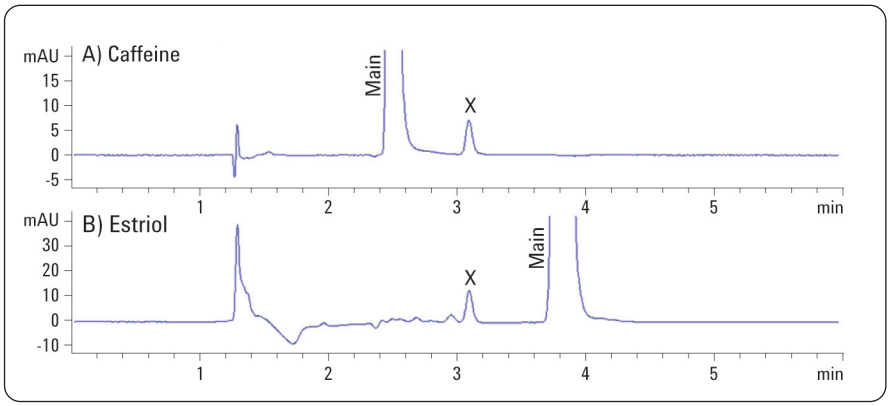


Figure 1
Isocratic separation of the impurity (0.05% w/w level) from the main component A) caffeine and B) estriol.

	Matrix	Caffeine		Estriol	
	Detection	UV 254 nm		UV 220 nm	
	Mode	Isocratic	Gradient	Isocratic	Gradient
t_R					
	mean (min)	3.140	3.315	3.120	3.667
	s (min)	0.002	0.003	0.001	0.001
	RSD (%)	0.077	0.098	0.047	0.023
Area					
r^2	0.01% to 0.50%	0.9988	0.9998	0.9973	0.9999
RSD	n=6 at 0.50%	0.81	0.26	0.14	0.38
RSD	n=6 at 0.05%	2.99	1.95	2.01	3.08
noise	mAU, 6*SD	0.06	0.08	0.12	0.13
S/N	at 0.01%	42	27	84	31
LOD	S/N > 10	0.003	0.004	0.001	0.003

Table 3
Validation results for isocratic and gradient methods.

and 0.9973 for thiourea in estriol (220 nm). RSDs at 0.05% level were 2.99% and 2.01%, respectively. The signal-to-noise ratios (S/N), measured at the 0.01% level, were respectively 42 and 84. Excellent retention time repeatability (RSD < 0.1%) was obtained.

Gradient Conditions

The study repeated the same analyses using a modifier gradient. Figures 2A and 2B show the chromatograms for the spiked samples at a 0.05% w/w level. The gradient time was 6 min in each case, but a different slope was used (Table 2).

The data show thiourea at 3.3 min in caffeine and at 3.7 min in estriol, illustrating good separation. Interestingly, another impurity in estriol is eluting closely after thiourea. This impurity eluted before thiourea in the isocratic method.

Table 3 shows the validated gradient methods and the results for this study as well. The linear regressions had r^2 values of 0.9998 (thiourea in caffeine, 254 nm) and 0.9999 (thiourea in estriol, 220 nm). RSDs at the 0.05% level were respectively 1.95% and 3.08%.

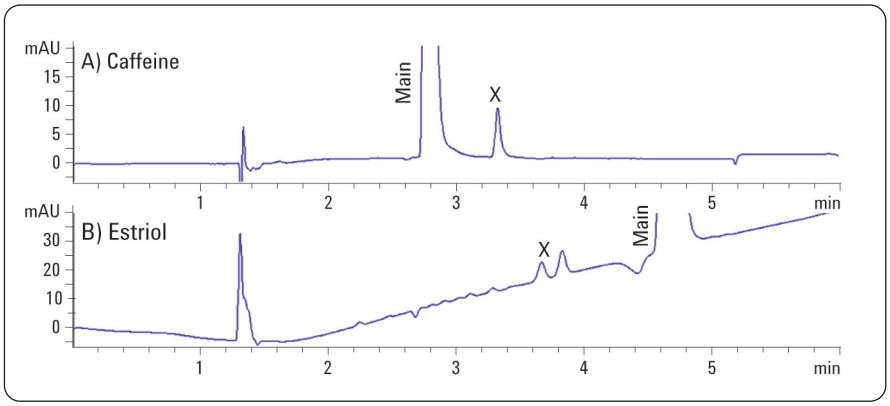


Figure 2
Gradient separation of the impurity (0.05% w/w level) from the main component A) caffeine and B) estriol.

The S/N ratios were calculated for the 0.01% (w/w) level as 27 for caffeine (254 nm) and 31 for estriol (220 nm). The sensitivity is slightly lower in gradient mode than in isocratic mode, especially using UV detection at 220 nm, due to the modifier gradient. However, in all cases, limits of detection (LODs) were below 0.01%.

Conclusion

This Application Note demonstrates the suitability of the Agilent 1260 Infinity Analytical SFC System for the detection of achiral low level impurities for pharmaceutical analysis.

The resulting data show excellent linearity, repeatability and sensitivity in both isocratic and modifier gradient modes. The low baseline noise resulted in LODs below 0.01% impurity versus the main solute (using a 5 mg/mL solution and 5 µL injection). This strongly indicates that the Agilent 1260 Infinity SFC System is a superior tool for standard SFC applications such as chiral analyses. It also shows that its application range can be easily expanded to a wide class of small molecules due to superior performance and sensitivity.

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