DIONEX

Application Note 195

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Determination of Verapamil Hydrochloride Purity Using the Acclaim PA Column

INTRODUCTION

Verapamil-based medications are prescribed for several heart and blood pressure indications. The fast-acting formulations (verapamil hydrochloride and Isoptin®) are taken for angina, as well as irregular heartbeat and high blood pressure. The United States Pharmacopeia (USP®) has a monograph method to determine verapamil hydrochloride purity. This method separates verapamil hydrochloride and verapamil-related compound B using HPLC with a C18 (USP designation L1) column.[1] There is a recent proposal to revise the method by using a L60 column to determine verapamil hydrochloride and verapamil-related compounds B and D. The existing method can not determine compound D under the prescribed eluent. The proposed method is time consuming (61 min), and requires a special column.[2]

In this application note, we describe a new method for the fast determination of verapamil hydrochloride and verapamil-related compounds A, B, and D, using a polar-embedded reversed-phase column, the Acclaim® PolarAdvantage (PA). The new method requires only about half the time of the proposed USP monograph method, provides significant eluent and therefore cost savings, and meets the resolution requirement.

EQUIPMENT

UltiMate® 3000 HPLC

HPG 3400 pump with SRD 3400 degasser

WPS 3000 TSL autosampler

TCC 3000 thermostatted column compartment

VWD-3400 UV-Vis detector

Chromeleon® 6.80 SP1 Chromatography Workstation

REAGENTS AND STANDARDS

Water, Milli-Q water from Milli-Q Gradient A10

Acetonitrile (CH_3CN), Fisher, HPLC grade

KH_2PO_4, reagent grade, (AR, analytical pure, grade in China)

H_3PO_4, reagent grade, (AR, analytical pure, grade in China)

Verapamil HCl (Sigma, CAS: 152-11-4), purity > 99.0%

Verapamil-related compound A (USP, P/N: 71130)

Verapamil-related compound B (USP, P/N: 71140)

Verapamil-related compound D (LGC Promochem GmbH, P/N: USP1711428)

PREPARATION OF REAGENTS AND STANDARDS

Prepare two solutions for testing, consistent with the requirements of the USP monograph method.

System suitability solution:

Prepare a mixture of verapamil hydrochloride, verapamil-related compound B, and verapamil-related compound D where each component has a concentration of 25 µg/mL. Add an additional component not described in the USP method, verapamil-related compound A, also at 25 µg/mL. Use this solution for method development.

Standard solution and test solution:

Prepare a 2.5 mg/mL standard solution of verapamil hydrochloride. To this solution add the three related compounds to achieve a final concentration of 2.5 µg/mL each, which serves as the test solution to simulate real samples.

CHROMATOGRAPHIC CONDITIONS

Column: Acclaim PA, 5 µm, 4.6 × 250 mm (P/N 061321)

Temperature: 35 °C

Inj. Volume: 5 µL

Mobile Phase: A: 20 mM KH_2PO_4 adjust pH to 3.0 with H_3PO_4

B: CH_3CN

Detection: Absorbance at 278 nm

Gradient Table:

Time	Flow Rate	Buffer (%)	CH_3CN (%)	Curve
0.0	1.0 mL/min	70	30	
4.0	1.0 mL/min	70	30	5
29.0	1.0 mL/min	45	55	5
29.5	1.0 mL/min	45	55	5
30.0	1.0 mL/min	70	30	5
35.0	1.0 mL/min	70	30	5

RESULTS AND DISCUSSION

Verapamil (Figure 1) with two aromatic rings is ideally suited for analysis by reversed-phase HPLC. Using the proposed USP monograph method as a starting point, we developed a much faster method that requires significantly less eluent. Figure 2 shows a separation of the system suitability solution described in the proposed revision to the USP monograph method with an addition of verapamil-related compound A. All four compounds are well resolved. The resolution of verapamil and verapamil-related compound B is >5.0 as required by the proposed method and >1.5 as required by the current USP monograph method.

Verapamil-related compound D is eluted, as required by the proposed USP method. Table 1 shows the repeatability for retention time and peak areas for five injections of the system suitability standard. The method described here requires only 35 min compared to the 61 min required by the proposed USP method. In addition to the 26 min savings, there is 56.5 mL of eluent savings per injection because the method described here is run at 1.0 mL/min rather than 1.5 mL/min. Chromatography of the test solution (Figure 3) shows that this method easily detects 0.1% quantities of verapamil-related compounds A, B, and D relative to verapamil at 2.5 mg/mL, making it ideal for verapamil purity analysis.

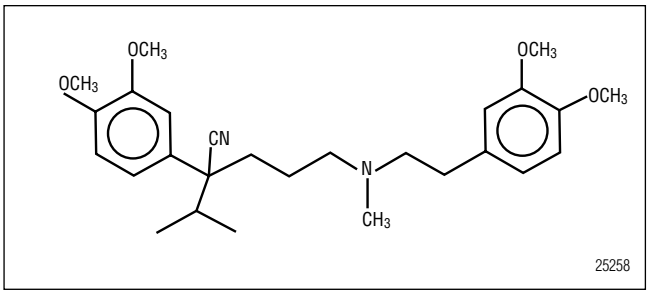

Figure 1. Structure of verapamil.

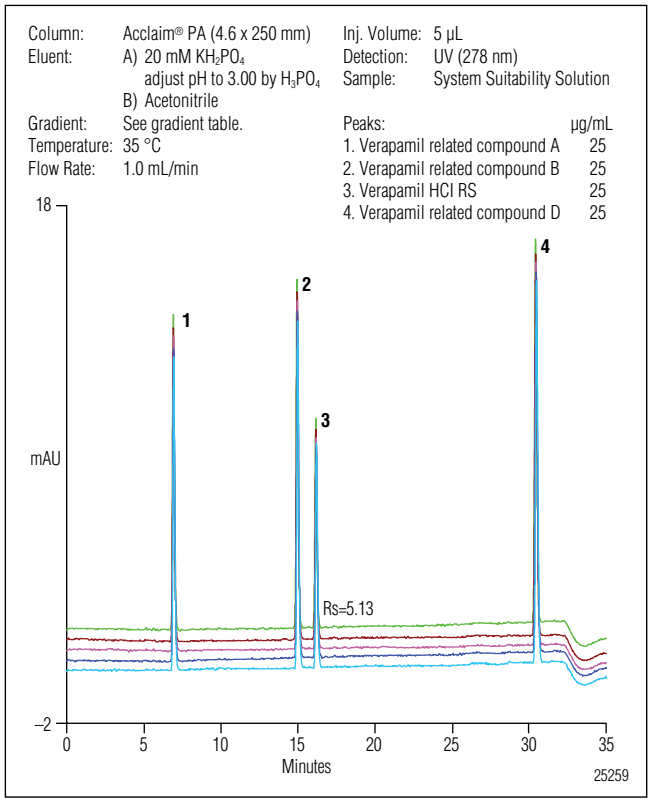

Figure 2. Overlay of five chromatograms of the system suitability solution.

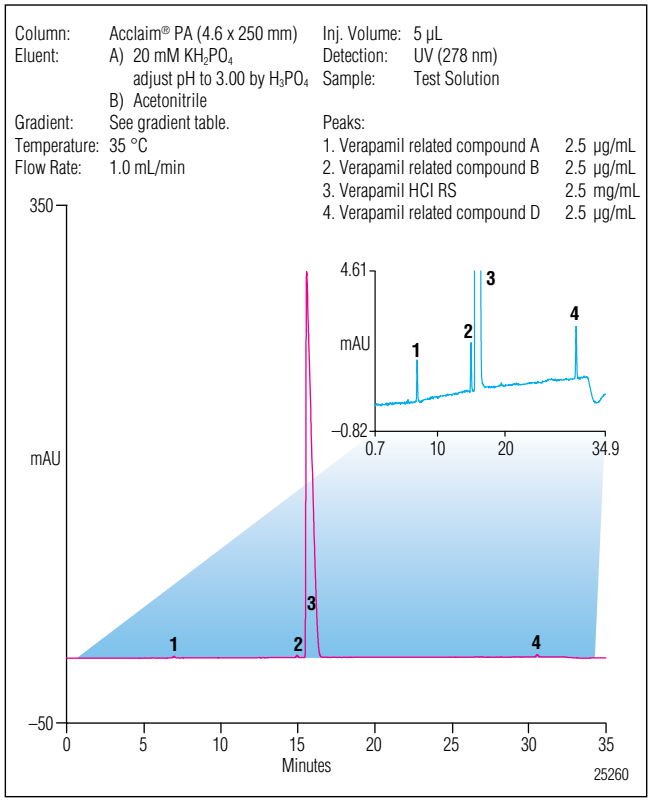

Figure 3. Chromatogram of the test solution.

Table 1. Repeatability of Retention Time and Peak Areas of Five Injections of the System Suitability Solution

RT (min)/ Area (mAU•min)	Verapamil Related Compound A		Verapamil Related Compound B		Verapamil HCl RS		Verapamil Related Compound D	
Injection 1	6.997	1.7798	15.084	15.084	2.0539	16.037	1.2225	2.553
Injection 2	6.991	1.7572	15.083	15.084	2.0654	16.305	1.2338	2.5308
Injection 3	6.99	1.7789	15.082	15.084	2.0771	16.301	1.2186	2.5278
Injection 4	6.989	1.7884	15.075	15.084	2.082	16.295	1.2391	2.54
Injection 5	6.984	1.7802	15.068	15.084	2.0538	16.29	1.228	2.5497
RSD	0.07	0.66	0.04	0.63	0.04	0.67	0.04	0.44

To achieve these improvements over the proposed USP monograph method, our method uses a polar-embedded reversed-phase column, the Acclaim PA, rather than an L60. No current L description suitably describes the resin in the PA column. It previously proved successful in improving the USP monograph method for nevirapine, which also prescribes a L60 column.[3] To shorten run time, achieve the best resolution, and work at a pH that will ensure the longest possible column life, the pH of the mobile phase is adjusted to 3.0 rather than the 7.2 pH recommended for the L60 column mobile phase.

Effect of mobile phase pH

During method development, testing the effect of pH 2.4 to 7.0 showed that lower pH provides higher resolution of verapamil-related compound B and verapamil. However, pH values lower than 3.1 did not significantly improve resolution. To meet the USP resolution requirement (> 5.0), a 250 mm column was required (same length as the L60). If a 150-mm column is preferred and a resolution of 5.0 between verapamil and verapamil-related compound B is not required, the 150-mm column will yield a resolution of about 4.0.

Figures 4 and 5 show the system suitability and test solutions analyzed at pH 2.9, 3, and 3.1. The chromatography shows that the method is rugged when challenged by small changes in pH.

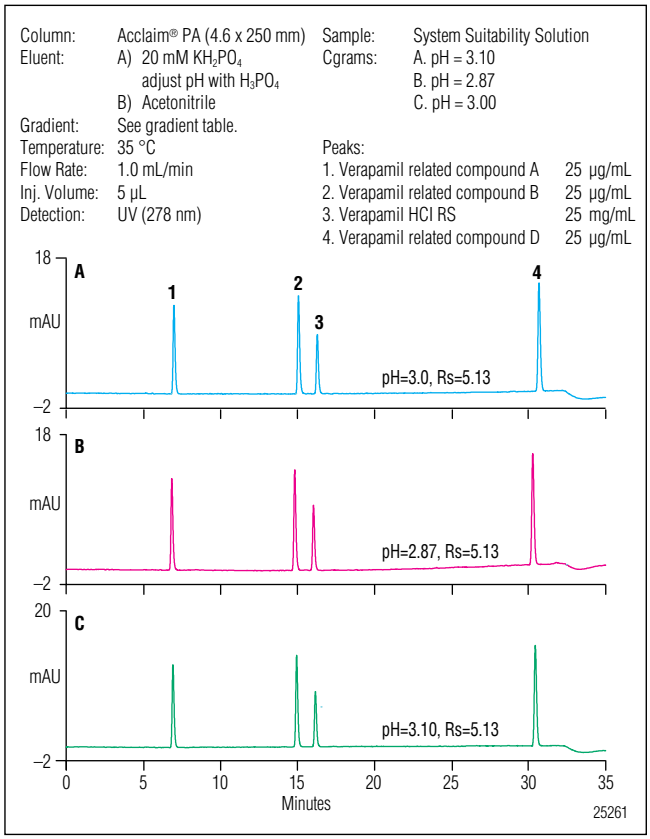

Figure 4. Overlay of chromatograms of the system suitability solution analyzed with different pH mobile phases. Peaks: 1) verapamil-related compound A; 2) verapamil-related compound B; 3) verapamil HCI RS, 4) verapamil-related compound D.

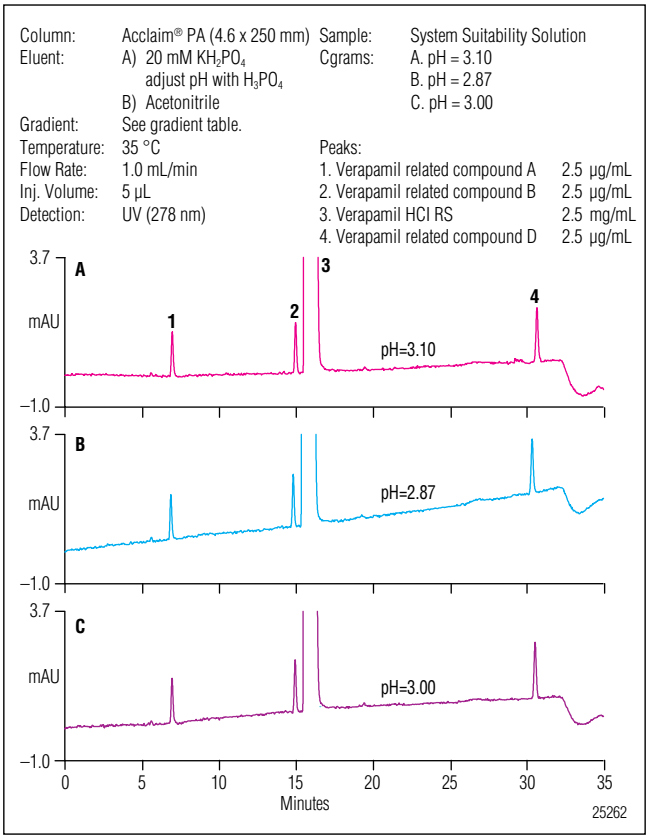

Figure 5. Analysis of the test solution with different pH mobile phases. Peaks: 1) verapamil-related compound A (2.5 µg/mL); 2) verapamil-related compound B (2.5 µg/mL), 3) verapamil HCI RS (2.5 mg/mL); 4) verapamil-related compound D (2.5 µg/mL).

REFERENCES

1. United States Pharmacopeia National Formulary USP30/NF25 (2007), 3454.
2. Pharmacopeial Forum (2006) 32(2) 389.
3. Dionex Application Note 180.

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