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Effective Determination of Pharmaceutical Impurities by Two Dimensional Liquid Chromatography

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APPLICATION BENEFITS

- Increase productivity through targeted 2DLC platform approach
- Improve data quality in the second dimension by utilizing At-column Dilution

WATERS SOLUTIONS

ACQUITY® UPLC® H-Class Bio System with 2D Technology

2DLC System preconfigured with At-column dilution (ACD) technique

CORTECS® UPLC C+18, 1.6 µm, 2.1 x 150 mm Column

ACQUITY UPLC BEH Phenyl 1.7 µm, 2.1 x 150 mm Column

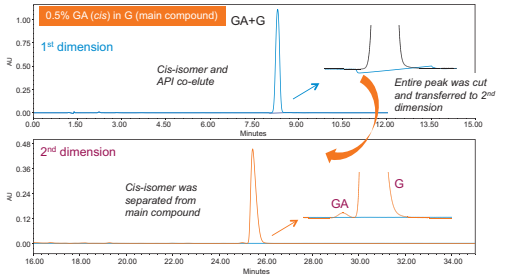
KEYWORDS

2DLC, Multi-dimensional chromatography, heartcut, At-column dilution, impurities, isomeric, USP, glimepiride

INTRODUCTION

Analysis of many pharmaceutical compounds often involves evaluation and quantification of impurities in the presence of an active pharmaceutical ingredient (API). Co-elution of the impurity with the API is a major challenge, particularly when other related compounds are also present in the sample. To address these challenges, a minimum of two methods are typically employed: a primary method to assess the majority of related compounds and a secondary method to address the impurities that may co-elute with API in the primary method. Alternatively, two-dimensional liquid chromatography (2DLC) offers a solution to combine these two analyses to effectively analyze all impurities in one chromatography system setup, saving analysis time and increasing productivity.

In this work, a targeted 2DLC platform is evaluated for a complete analysis of an API and related impurities, including an impurity that co-elutes with the API. In the first dimension, a reversed phase (RP) method is used to separate related impurities from the peak that contains the main compound of interest. This peak is then transferred to the second dimension to separate a co-eluting impurity from the main compound. The approach uses a heartcut process combined with trap and elute. At-column Dilution (ACD) is also implemented for improved chromatographic performance in the second dimension for a targeted 2DLC platform, "Heartcut-ACD-Trap-Elute". The work flow and strategy for the targeted 2DLC platform is described including the rationale of adding both ACD and a trap column. Data quality and the impact of the targeted approach in the second dimension are also explained. Using this proposed targeted 2DLC approach, the quantification of low-level impurities in pharmaceutical drug substances, including geometric isomers will be shown.



In the 1st dimension, cis-isomer impurity (GA) and the main compound (G). The main peak was transferred to the 2nd dimension, where the co-eluted compounds were separated.

ACQUITY UPLC H-Class Bio 2DLC System.

EXPERIMENTAL

Method conditions

LC conditions

LC system:	ACQUITY UPLC H-Class Bio System with 2D Technology	
Sample manager:	ACQUITY UPLC H-Class bio Sample Manager – FTN (SM-FTN)	
Column manager:	ACQUITY UPLC Column Manager (CM)	
	1st dimension	**2nd dimension**
Pump:	ACQUITY UPLC Quaternary Solvent Manager (QSM)	ACQUITY UPLC Binary Solvent Manager (BSM)
Detectors:	ACQUITY UPLC Photodiode Array (PDA)	ACQUITY UPLC Tunable Ultra-Violet (TUV)
Absorption wavelength:	228 nm	228 nm
Column:	CORTECS UPLC C_{+18}, 1.6 µm, 2.1 x 150 mm (p/n 186007117)	ACQUITY UPLC BEH Phenyl 1.7 µm, 2.1 x 150 mm (p/n 186003378)
Column temp.:	30 °C	35 °C
Flow rate:	0.2 mL/min	0.25 mL/min
Mobile phase A:	8.3 mM phosphate buffer, pH 2.1~2.7	
Mobile phase B:	Acetonitrile	73% 50 mM phosphate buffer (pH 7.00) containing 1% triethyl amine/18% acetonitrile/9% tetrahydrofuran (v/v)
Isocratic:	50% B	100% B
Trap column:	XBridge® C8, Direct Connect HP, 2.1 x 30 mm, 10 µm (p/n 186005233)	XBridge® C8, Direct Connect HP, 2.1 x 30 mm, 10 µm (p/n 186005233)
Sample temp.:		Ambient
Injection volume:		3.3 µL

At-column dilution (ACD)

Pump:	ACQUITY UPLC Isocratic Solvent Manager (ISM)
Flow rate:	0.8 mL/min
Mobile phase:	50 mM phosphate buffer (pH 7.00) containing 1% triethyl amine

Chromatography data system

Empower® 3, Feature Release 2

Sample description

Glimepiride (G) and glimepiride related compounds were purchased from USP (Rockville, MD 20852). The glimepiride related compounds include glimepiride *cis*-isomer or related compound A (GA), glimepiride sulfonamide or related compound B (GB), glimepiride urethane or related compound C (GC), and glimepiride 3-isomer or related compound D (GD).

Glimepiride drug substance was obtained from Alibaba.com (Shanghai, China). All stock solutions of glimepiride drug substance, glimepiride standard and glimepiride related compounds were prepared with acetonitrile/water (80/20, v/v) at 0.2 mg/mL.

RESULTS AND DISCUSSION

2D CHROMATOGRAPHIC SYSTEM FOR "HEARTCUT-ACD-TRAP-ELUTE" STRATEGY

For this configuration, the ACQUITY UPLC H-Class Bio System with 2D technology is configured with ACQUITY UPLC modules and the ACQUITY UPLC column manager consisting of two, 2-position/6-port valves (Figure 1). With both valves in position 2, the flow from the QSM and BSM are independent of each other. This allows for independent control of the effluent on the 1st dimension column and the 2nd dimension column.

The work flow and key events associated with the steps in the peak transfer process can be illustrated through a series of valve switches. At the injection start, the sample is injected onto the 1st dimension column with the effluent going to the 1st dimension detector (Figure 2A). When the peak of interest begins to elute off the column, the 1st dimension valve (left valve) switches positions and diverts the effluent to a trap column in the 2nd dimension (Figure 2B). The trap column acts like a "sample loop" to temporarily store the sample. However, unlike a sample loop, which has a defined volume, the trap column can retain a peak over a range of volumes. This approach facilitates quantification in 2nd dimension when an entire large peak needs to be transferred to the 2nd dimension to separate co-elutions. The use of trap column also minimizes system pressure as compared to directly transferring the sample from the 1st dimension column to the 2nd dimension column. The selection of trap column depends on the analyte of interest.

To address the challenges that may arise from strong solvent in the sample, at-column dilution (ACD) is performed by an addition of a third pump. Prior to the sample transfer to the trap column, ACD is used to dilute the organic composition of effluent. This independent pump allows the peak "diluent" to be optimized in order to retain and focus the sample on the trap column. After the sample is trapped, the 1st dimension valve returns to its initial position and the 2nd dimension valve switches positions to back flush the sample from the trap column onto the 2nd dimension column (Figure 2C).

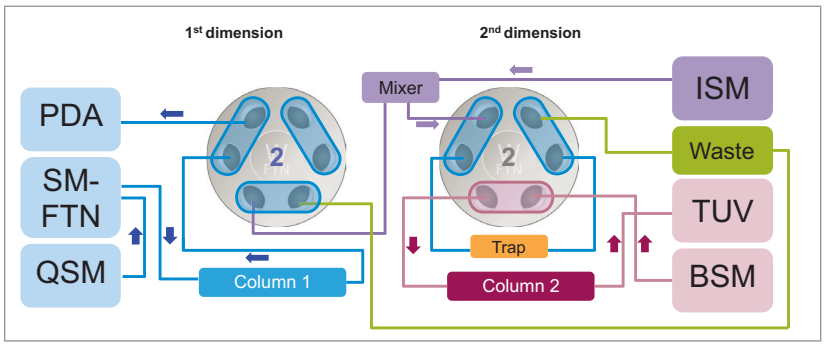


Figure 1. Plumbing diagram of the ACQUITY UPLC H-Class Bio System with 2D Technology featuring "Heartcut-ACD-Trap-Elute".

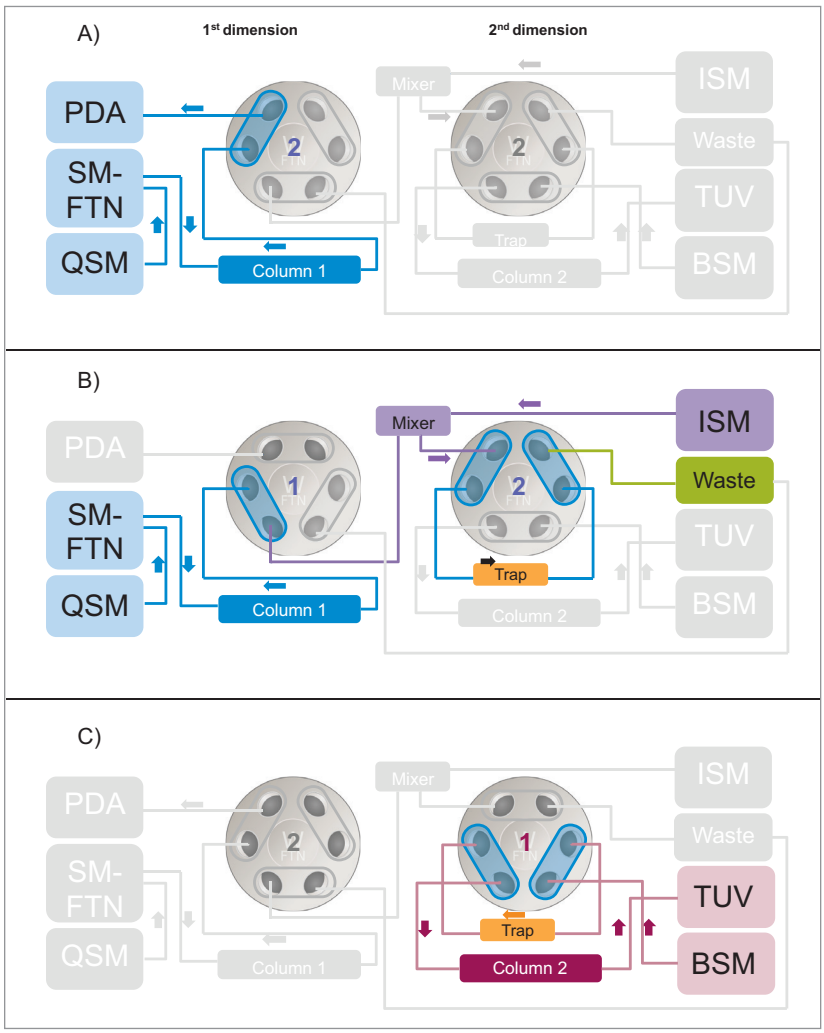


Figure 2. Work flow of targeted 2DLC "Heartcut-ACD-Trap-Elute" system.

A) Step 1: At injection start, sample is injected onto the 1st dimension column and effluent goes to the 1st dimension detector.

B) Step 2: Heartcut from the 1st dimension to trap, 1st dimension valve switches positions, which diverts effluent (sample) from the 1st dimension to the trap column; an ISM pump is used to dilute the organic composition of the effluent prior to the trap column – at-column dilution (ACD).

C) Step 3: Back-flush elute to 2nd dimension column, 1st dimension valve is returned to its initial position; 2nd dimension valve switches positions, which results in back-flush of sample from trap to the 2nd dimension column then to the 2nd dimension detector.

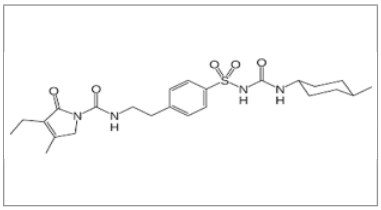
To accomplish the above work flow of "Heartcut-ACD-Trap-Elute", plumbing, pump and the valves events need be transcribed into an instrument method. Table 1 lists an example of an instrument method that consolidated individual module parameters, pumps and valves events. The peak transfer (heartcut) window, which includes the start and end of the peak, was marked with asterisks.

Time (min)	QSM		CM		BSM		ISM
	Flow (mL/min)	B%	Left valve	Right valve	Flow (mL/min)	B%	Flow (mL/min)
0.00	0.2	50	2	2	0.25	100	0.1
2.00							0.8
7.85*			1				
8.66*			2				
8.86				1			0.8
9.00							0
35.00	0.2	50			0.25	100	

Table 1. An example of an instrument method with pump parameters and valve events for "Heartcut-ACD-Trap-Elute". The start and stop time of the "heartcut" window are marked with asterisks.

USP RELATED COMPOUNDS ANALYSIS IN THE FIRST DIMENSION

Glimepiride (G), a sulfonylurea antidiabetic drug to treat type 2 diabetes, is an isomeric compound, with the API in the *trans* form, an isomeric impurity in the *cis* form and an additional isomeric impurity in the meta-isomer form (also called 3-isomer). For related impurity analysis, the USP monograph consists of two different tests: a normal phase HPLC method to analyze the *cis*-isomer impurity, and a RP-HPLC for the other related compounds.[1] The USP limits for the related compounds vary from 0.1 to 0.8% (Table 2).



Chemical structure of glimepiride (G).

Name	Chemical	Limit (%)
G related Compound A (GA)	G *cis*-isomer	0.8
G related Compound B (GB)	G sulfonamide	0.4
G related Compound C (GC)	G urethane	0.1
G related Compound D (GD)	G 3-isomer	0.2

Table 2. USP limits for related compounds of glimepiride (G).[1]

In order to improve throughput, the USP monograph for organic impurities method was scaled from HPLC to UPLC in the 1st dimension. The scaled conditions were determined using the Waters Column Calculator with changes in flow rate to improve the separation of 3-isomer (GD) from API (G). A solid-core column (CORTECS UPLC C_{18+}, 1.6 µm) was selected to take advantage of the improved efficiency and lower pressures of superficially porous columns. The overlay of multiple injections of the system suitability standards (Figure 3) shows the repeatability of the method in the 1st dimension. For all compounds the retention time repeatability was <0.2% RSD and the peak area repeatability was <1% RSD. Analysis of the glimepiride drug substance in the 1st dimension showed the presence of 0.3% GB and 0.07% GD, both of which were below the USP limits (not shown).

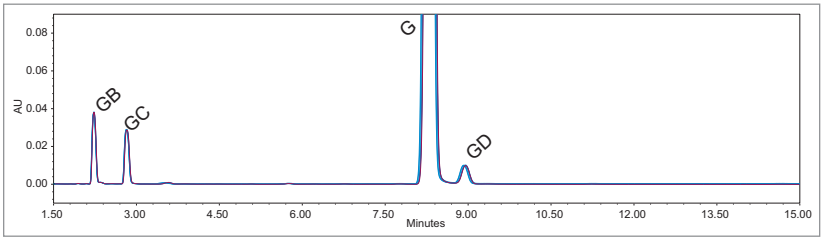


Figure 3. Overlay (n=6) of system suitability standards of related compounds of glimepiride in the 1st dimension.

Peak Name	GB	GC	G	GD
Retention time (RSD%)	0.11%	0.14%	0.17%	0.18%
Peak area (RSD%)	0.96%	0.31%	0.05%	0.45%

Table 3. Results of system suitability standards (n=6) for related compounds analysis in the 1st dimension.

CIS-ISOMER IMPURITY ANALYSIS IN THE SECOND DIMENSION

Although related compounds of glimepiride were analyzed in the 1st dimension, the *cis*-isomer co-eluted with the main compound. In order to separate the impurity from API, the entire peak was cut and diverted to the 2nd dimension. In the 2nd dimension, a UPLC method scaled from a previously published RP-HPLC method was used.[2] An ACQUITY UPLC BEH Phenyl 1.7 µm, 2.1 x 150 mm column was selected for its unique selectivity and reduced runtime. Figure 4 illustrates this heartcut process in the 1st dimension and the separation of low level of *cis*-isomer (0.5%) from API in the 2nd dimension. The repeatability of the heart-cutting process was demonstrated by six replicates of injections of glimepiride containing 0.5% *cis*-isomer. Retention time repeatability was <0.1 %RSD and peak area repeatability was <0.5% RSD for the low level isomeric impurity and glimepiride (see Table 4).

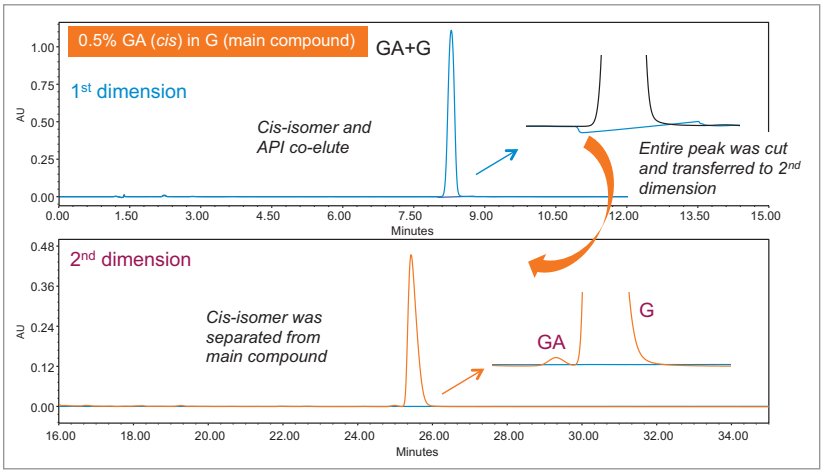


Figure 4. In the 1st dimension, cis-isomer impurity (GA) and the main compound (G) (top chromatogram). The main peak was transferred to the 2nd dimension, where the co-eluted compounds were separated (bottom chromatogram).

Peak Name	GA	G
Retention time (min)	24.995	25.437
RSD%	0.04%	0.04%
Area	43496	7403064
RSD%	0.46%	0.24%

Table 4. Statistical evaluation of the analysis of 0.2 mg/mL glimepiride (G) solution spiked with 0.5% cis-isomer (GA) in 2nd dimension.

To quantify the *cis*-isomer in the 2nd dimension, a calibration curve using *cis*-isomer (GA) was established. Figure 5 shows the overlay of GA standards at different concentrations. The calibration curve was linear (R^2>0.995) over the range (0.1–2.5% of the API) investigated. The LOQ is 0.1% (s/n >5) and the LOD is 0.07% (s/n >3) of *cis*-isomer in glimepiride. Using the established calibration curve, the *cis*-isomer impurity in glimepiride drug substance was quantified against the GA calibration curve. The results showed the *cis*-isomer impurity was below the LOQ (0.1%) but above the LOD (0.07%) in the glimepiride drug substance (Figure 6).

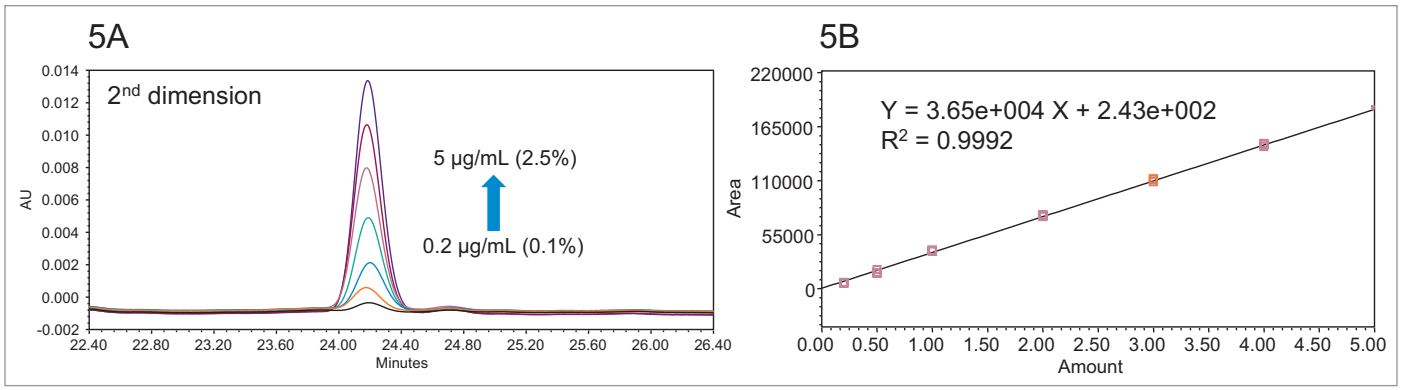


Figure 5. A) Overlay of cis-isomer (GA) standards with different concentrations, and B) calibration curve of GA in the 2nd dimension.

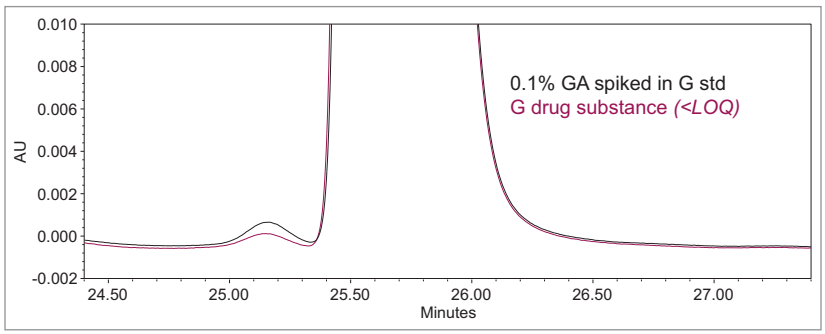


Figure 6. Overlay of chromatograms in the 2nd dimension: glimepiride (G) drug substance and glimepiride standard spiked with 0.1% cis-isomer (GA).

USE OF AT-COLUMN DILUTION (ACD) FOR IMPROVED RESOLUTION

ACD is a technique used to address strong solvent effects that result in poor retention and chromatographic peak distortions.[3,4] In 2DLC, these effects are most noticeable in RPLC-RPLC configuration when the "heartcut" contains a high percent of organic. By adding an additional pump to dilute the organic composition of the "cut" prior to 2nd dimension column, the strong solvent effect can be reduced, resulting in improved retention and peak shape for the analyte. This technique, ACD, can be preconfigured as part of the Waters 2DLC System.

Figure 7 compares the separation of *cis*-isomer from glimepiride in the 2nd dimension with ACD and without ACD. The dilution factor of ACD is determined by the proportion of two variables: the total flow rate (FR_T) and flow rate from the 1st dimension pump (FR_1). In this case, flow rate from the 1st dimension is 0.2 mL/min and the flow rate from diluting pump is 0.8 mL/min. Thus, the dilution factor can be calculated by dividing the sum of the flow rate by that of the 1st dimension pump or by:

$$FR_T/FR_1 = (0.8\ mL/min + 0.2\ mL/min)/0.2\ mL/min = 5$$

This indicates the sample is diluted 5 x by the ACD pump. For example, when the "cut" in the 1st dimension contains 50% organic, it reduced 5-fold to 10% organic using ACD (Figure 8). This technique enabled the separation of *cis*-isomer impurity from main compound.

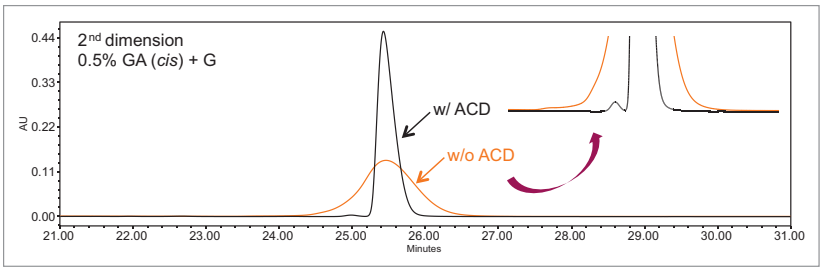


Figure 7. Waters ACD improves the separation of cis-isomer (GA) from glimepiride (G) in the 2nd dimension.

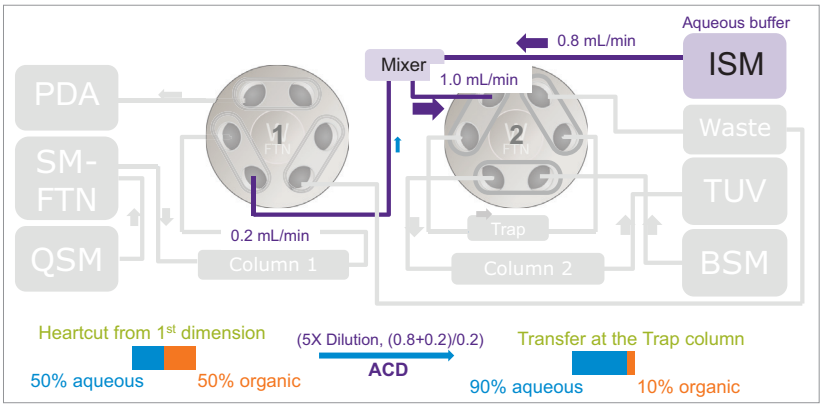


Figure 8. Principles of Waters ACD applied to transferring "Heartcut" from the 1st dimension to the 2nd dimension. 0.2 mL/min 1st dimension flow rate and 0.8 mL/min ISM (aqueous mobile phase) flow rate results in a 5x dilution of the "Heartcut" from the 1st dimension.

CONCLUSIONS

The analysis of pharmaceutical drugs and their impurities can be challenging, particularly when geometric isomer impurities are present. To address these challenges, a 2D integrated solution can enable both analyses to be performed on a single system setup. This allows for increased productivity with reliable quantitation for both separations.

The ACQUITY UPLC H-class Bio System with 2D technology featuring "Heartcut-ACD-Trap-Elute" addresses these challenges. This solution provides a RPLC separation in the 1st dimension to separate related compounds of API, and *cis*-isomer separation in the 2nd dimension. The reliability of the heartcutting process is shown by the retention time and peak area reproducibility of the low level isomeric impurity in the presence of main compound. A linear calibration curve (R^2>0.995) of *cis*-isomer impurity was also established in the 2nd dimension. The example, a pharmaceutical drug, demonstrates the capability of Waters targeted 2D system to quantify low level impurities in both dimensions at levels that meet the USP requirements.

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