

# Quantification of caffeine with the AZURA® Educational system and ClarityChrom software



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## SUMMARY

The AZURA® HPLC Educational System allows an easy and fast introduction to liquid chromatography (HPLC, high pressure liquid chromatography) and promotes a deeper understanding of this separation method. A simple example is given describing the determination of a sample containing caffeine, paracetamol and theophylline.

## INTRODUCTION

Caffeine and paracetamol are widely-used pharmaceutical components. Both substances are present as ingredients in many analgesics. Hence, they are often determined simultaneously in routine analysis such as quality control. Theophylline, a substance chemically closely related to caffeine, is used to serve as an internal standard. [1] To analyze these three components, the AZURA Educational System was used providing isocratic elution HPLC

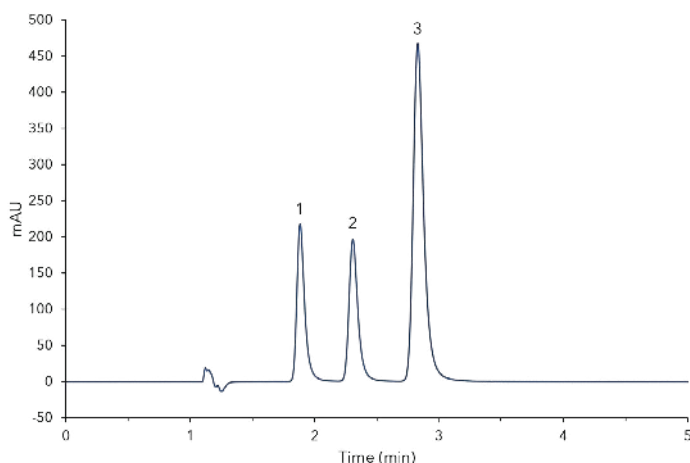
in combination with UV detection. The samples were injected via a manual injection valve. Based on the KNAUER AZURA Compact series, this system layout represents an easy and convenient solution for the current application. ClarityChrom is an easy-to-use Chromatography Data System for workstations. The Educational System license is exclusively bundled with the AZURA Educational System.

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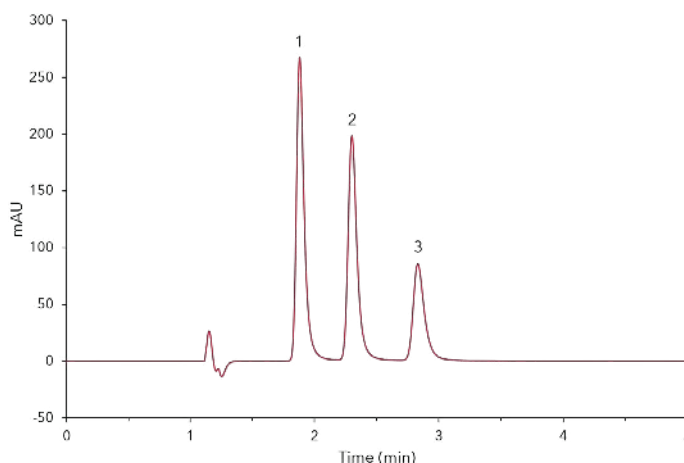
## RESULTS

At first, stock solutions are prepared from caffeine, paracetamol and theophylline. The initial weight of the substances should be about 100 mg. However, it is important to note the exact sample weight to obtain accurate results for the quantitative analysis. All standards were dissolved and sonicated to yield stock solutions of approximately 10 mg/mL. To identify the individual substances directly by HPLC, the substances are diluted with water (Table 1, Additional Materials and Methods). Secondly, a single calibration solution is prepared from the caffeine and paracetamol stock solution. For this purpose, 50  $\mu$ L of the caffeine stock solution and 50  $\mu$ L of the paracetamol stock solution are combined and diluted with water to a final volume of 5 ml. For the current application, five dilution levels (Tab. 2, Additional Materials and Methods) have been prepared. Furthermore, 100  $\mu$ L of the theophylline stock solution are diluted

with water to a final volume of 1 mL. Subsequently, a volume of 20  $\mu$ L of this solution is added to standard 1 - 5. Fig 1 shows the chromatogram of the calibration standard at level 4 (60 $\mu$ g/mL). The peaks are baseline separated in less than 5 minutes. Fig 2 shows the measurement of an analgesic sample. Therefore, analgesic tablets containing paracetamol and caffeine were chosen. The internal standard theophylline was added to the sample according to the preparation of calibration solutions. A concentration of 53 mg caffeine was calculated for the sample. This amount refers to the weight of one tablet. Relating to the package insert, the analgesic should contain 50 mg caffeine per tablet. The deviation of the measured and proclaimed value might result from differing calibrations and/or measurement errors. For the detailed preparation of standards, sample and calibration please see application VSP0018.



**Fig. 1** Chromatogram of standard solution 4, 1) paracetamol, 2) theophylline (IS), 3) caffeine



**Fig. 2** Chromatogram of analgesic sample, 1) paracetamol, 2) theophylline (IS), 3) caffeine

## MATERIALS AND METHODS

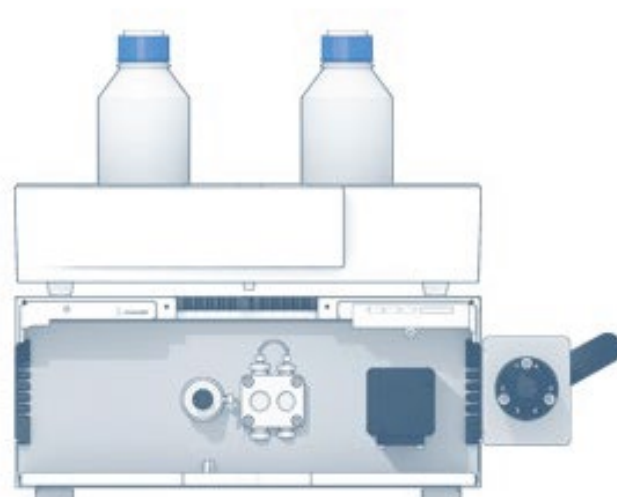
For the determination of caffeine and paracetamol the AZURA® Educational system was used, which combines a P 4.1S pump, an UVD 2.1S and a manual injection valve in just one AZURA assistant. The flow rate was set to 0.8 mL/min at ambient temperature. The wavelength was set to 273 nm with a data rate of 20 Hz and a time constant of 0.05 s. 10 µL of the standards and samples were injected. The isocratic method ran for 5 minutes with a mixture of methanol and water 40:60 (v/v). The column in a dimension 125 x 4 mm ID with precolumn was filled with Eurospher II 100-5 C18 silica. For the data acquisition the ClarityChrom software was used.

## CONCLUSION

The AZURA HPLC Educational System provides both, a qualitative and quantitative analysis of caffeine from different chemical samples. The system is compact, very simple to operate and can be ideally used for practical training courses. The chromatography data system ClarityChrom offers an intuitive system configuration, control and the evaluation of data. More detailed information on hardware and software is available by videos and manuals which will be delivered with the system. The AZURA Educational system video tutorials are available on our website.

## REFERENCES

[2] Entry: internal standard. In: IUPAC Compendium of Chemical Terminology (the "Gold Book"). doi:10.1351/goldbook.i03108.



## ADDITIONAL RESULTS

**Tab.A1** Initial weight and dilution of stock solutions

Substance	Initial weight (mg)	Final conc. stock solution (mg/mL)	Final conc. diluted solution (µg/mL)
Caffeine	100.0	10.0	100.0
Theophylline	99.3	9.9	99.3
Paracetamol	98.7	9.9	98.7

**Tab.A2** Caffeine standards 1 to 5

Caffeine standard	Projected caffeine conc.(V = 1 mL) (µg/mL)	Actual caffeine conc. (V = 1.02 mL) (µg/mL)
1	5	4.9
2	20	19.6
3	40	39.2
4	60	58.7
5	80	78.4

## ADDITIONAL MATERIALS AND METHODS

**Tab.A3** Method parameters

Eluent	Methanol:Water 40:60 (v/v)		
Gradient	isocratic		
Flow rate	0.8 mL/min	System pressure	approx. 115 bar
Column temperature	RT	Run time	5 min
Injection volume	10 µL	Injection mode	Full loop
Detection wavelength	273 nm	Data rate	20 Hz
		Time constant	0.05 s

**Tab.A4** System configuration & data

Instrument	Description	Article No.
System	AZURA® Educational system	<a href="#">671101100</a>
Column	Eurospher II 100-5 C18, Vertex Plus 125 x 4 mm ID with precolumn	<a href="#">12WE181E2J</a>
Software	ClarityChrom® 7.2 - Educational License	<a href="#">A1672-11</a>



AZURA® Educational system

## RELATED KNAUER APPLICATIONS

**VSP0016** - Quantification of caffeine with the AZURA® Educational system and Mobile Control Software

**VSP0018** - Preparation of calibration and samples for the quantification of caffeine with the AZURA® Educational system

**VSP0019** - HPLC Basics - principles and parameters