

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

Juliane Böttcher, Mareike Margraf, Kate Monks; applications@knauer.net
KNAUER Wissenschaftliche Geräte GmbH, Hegauer Weg 38, 14163 Berlin; www.knauer.net



SUMMARY

The AZURA® Educational system allows an easy and fast implementation of 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 and paracetamol.

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. Theophylline, a substance chemically closely related to caffeine, is used to serve as an internal standard. [1] To analyze the components, the KNAUER HPLC Educational system is used providing isocratic elution

HPLC in combination with UV detection. The samples are 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. The determination of a sample containing caffeine and paracetamol is a typical example from applied research for the implementation of the KNAUER HPLC Educational system.

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

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 (Tab A1, Additional Materials and Methods). Secondly, a single calibration solution is prepared from the caffeine and paracetamol stock solution. For this purpose, 50 μ L of the caffeine stock solution and 50 μ 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 A2, Additional Materials and Methods) have been prepared. Furthermore, 100 μ L of the theophylline stock solution are diluted

with water to a final volume of 1 mL. Subsequently, a volume of 20 μ L of this solution is added to standard 1 - 5. Fig 1 shows the chromatogram of the calibration standard at level 4 (60 μ 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 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.

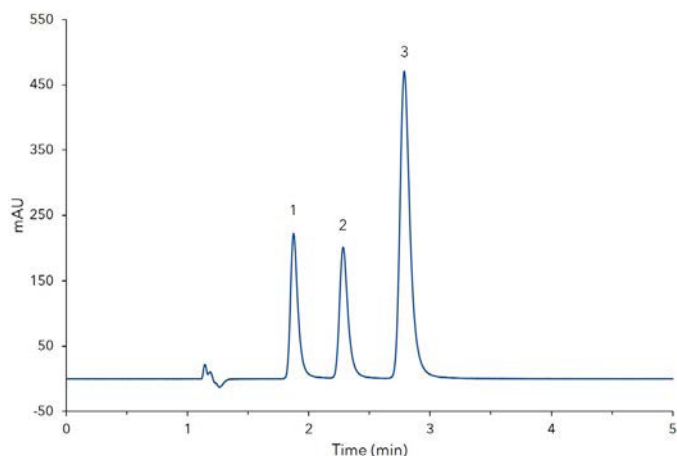


Fig. 1 Chromatogram of standard solution 4, 1) paracetamol, 2) theophylline (IS), 3) caffeine

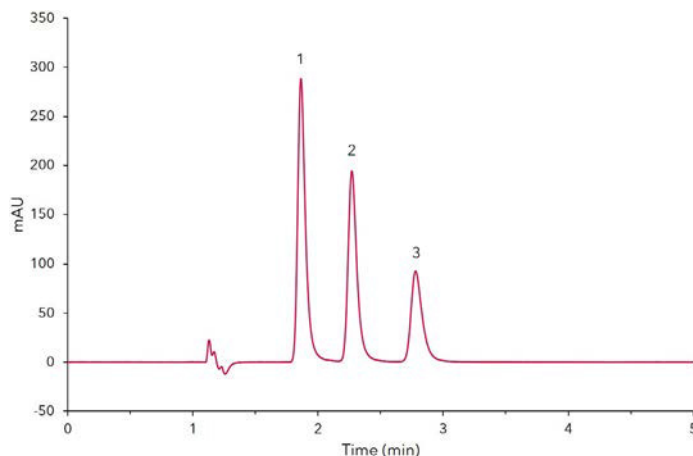


Fig. 2 Chromatogram of analgesic sample, 1) paracetamol, 2) theophylline (IS), 3) caffeine

MATERIALS AND METHODS

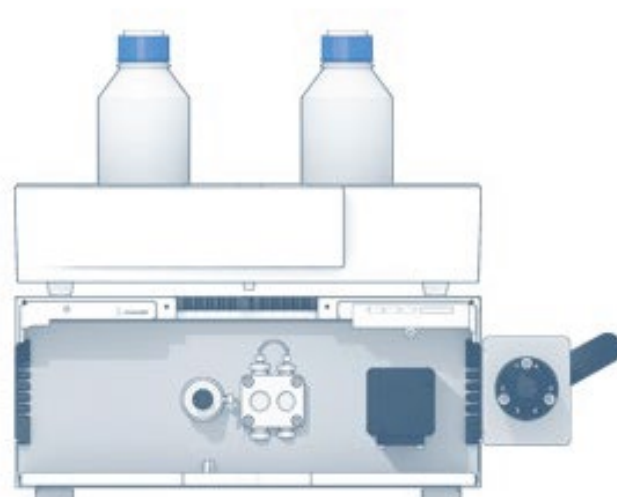
For the determination of caffeine and paracetamol the AZURA Educational system was used, which combines a P 4.1S pump, an UVD 2.1S and a manual injection valve in just one AZURA assistant. The flow rate was set to 0.8 ml/min at ambient temperature. The wavelength was set to 273 nm with a data rate of 20 Hz and a time constant of 0.05 s. 10 μ L 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 Mobile Control Chrom software was used.

CONCLUSION

The KNAUER HPLC Educational System provides both, a qualitative and quantitative analysis of caffeine from different chemical probes. The system is compact, very simple to operate and can be ideally used for practical training courses. The Mobile Control Chrom is an intuitive and cost-effective software solution for controlling and monitoring your AZURA devices and systems. In addition, you can acquire data from AZURA detectors allowing simple measurements.

REFERENCES

[1] 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	671101100
Column	Eurospher II 100-5 C18, Vertex Plus 125 x 4 mm ID with precolumn	12WE181E2J
Software	Mobile Control Chrom	A9608



AZURA® Educational system

RELATED KNAUER APPLICATIONS

VSP0017 - Quantification of caffeine with the AZURA® Educational system and ClarityChrom software

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

VSP0019 - HPLC Basics - principles and parameters