Science Together

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

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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

The following descriptions are necessary to perform the measurements shown in KNAUER application notes VSP0016 and VSP0017. A detailed procedure for the preparation, dilution, and calculation of standard solutions used for calibration and an analgesic sample will be executed here. This guidance is suitable for data acquisition with either Mobile Control or ClarityChrom.

CALIBRATION PREPARATION

At first, individual stock solutions were prepared from caffeine, paracetamol, and theophylline. The initial weight of the substances should be about 100 mg (NOTE: for subsequent quantification it was important to record the exact initial weight). The substances were dissolved in 10 mL of methanol and sonicated to yield stock solutions of approx. 10 mg/mL. To identify the individual substances directly by HPLC, the substances were then diluted 1:100 with water. **Tab. 1** shows exemplary initial weights and dilutions of the stock solutions.
Secondly, a single calibration solution was prepared from the caffeine and paracetamol stock solutions. For this purpose, 50 µL of the caffeine stock solution and 50 µL of the paracetamol stock solution were combined and diluted with water to a final volume of 5 mL (1:100 dilution). Thus, each individual substance had a concentration of approx. 100 µg/mL.

Tab. 1 Initial weight and dilution of stock solutions

Substance	Initial weight (mg)	Final conc. stock solution (mg/mL)	Final conc. diluted solution (µg/mL)
Caffeine	99.3	9.9	99.3
Theophylline	113.2	11.3	113.2
Paracetamol	107.7	10.7	107.7

For HPLC analysis, the dilution levels of the calibration solution should cover a range from 5-80 µg/mL. To ensure a correct measurement, at least four different dilution levels should be achieved. In this application, five dilution levels were prepared. The concentration of the calibration levels is shown in **Tab. 2**. The corresponding solutions were named standard 1 to 5. An additional calibration solution of the internal standard becomes necessary for quantitative HPLC analysis. For this purpose, 100 µL of the theophylline stock solution were diluted with water to a final volume of 1.00 mL (1:10 dilution, concentration approx. 1 mg/mL). Subsequently, a volume of 20 µL (final concentration approx. 20 µg/mL) of this solution wass added to the standard solutions 1 to 5. Since, by the addition of the internal standard the final volume increased by 20 µl, it was important to calculate the concentration of caffeine in the final volume of 1.02 mL (column 3, **Tab. 2**).

Tab. 2 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	19.5
3	40	38.9
4	60	58.4
5	80	77.8

INFOBOX: INTERNAL STANDARD

In chromatography an internal standard represents a compound which is added to a sample in a known concentration. It is used to facilitate the qualitative identification and/or quantitative determination of the sample components. An internal standard must be very similar but not identical to the chemical species of the analyte. Moreover, it should not occur in the investigated sample. [1]

CALIBRATION CALCULATION

To calibrate the system, the standard solutions were injected into the system and the peak areas were analyzed. Each standard solution was injected three times to ensure sufficient data acquisition.

Depending on which software is used for the data acquisition, the calculation of calibration is different. The ClarityChrom HPLC software correlates the peak areas of the standard solutions and the peak area of the internal standard for each concentration (method: ISTD calibration curve). When Mobile Control Chrom is used, the calibration curve is generated with the help of e.g. MS Office Excel (exemplary ISTD calibration curve see **Fig. 1**). With the Mobile Control Data Viewer the peak areas can be displayed but a calibration cannot be done automatically.

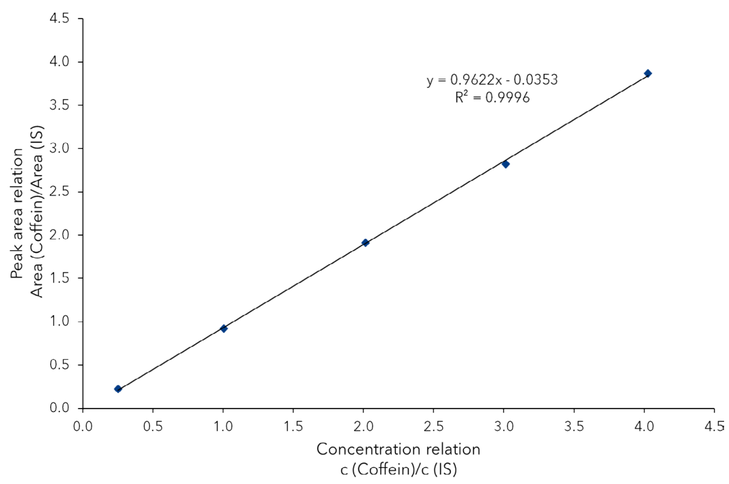


Fig. 1 Calculated ISTD calibration curve of caffeine (Mobile Control)

SAMPLE PREPARATION

A solid sample (tablet) of an analgesic product was crushed with a mortar to fine powder. Then approximately 100 mg of the homogenised sample were weighed and the weight was registered (important for quantitative analysis). The sample was then dissolved in 10 mL methanol. Thereafter, the sample was filtered through a syringe filter with a pore size of 0.45 µm. Subsequently, the filtered sample was diluted 1:100 with water. 1 mL of this solution was transferred to an appropriate vessel (vial). Similarly, to the standard solutions, the internal standard theophylline (20 µL, final concentration approx. 20 µg/mL) was added to the sample solution. After proper mixing of the sample solution, it was ready for HPLC analysis.

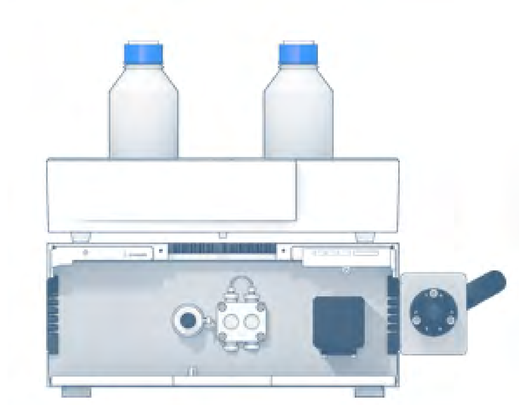
MATERIALS AND METHODS

The analytical parameters for determination are described in KNAUER application notes VSP0016 and VSP0017.

REFERENCES

[1] Entry: internal standard. In: IUPAC Compendium of Chemical Terminology (the “Gold Book”). doi:10.1351/goldbook.I03108.

Additional information



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ADDITIONAL MATERIALS AND METHODS

Tab. A1 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. A2 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	ClarityChrom 7.2 – Educational License Mobile Control Chrom	A1672-11 A9608

AZURA® Educational system

RELATED KNAUER APPLICATIONS

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

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

VSP0019 - HPLC Basics – principles and parameters