Determination of Caffeine in Energy Drinks

Introduction

Energy drinks are a type of beverage which should stimulate the organism. Therefore often caffeine is used, but also can contain taurine. Further components are sugar, water, dyes and aromatics. Caffeine is the most consumed psychoactive drug in the world. Belonging to the group of alkaloids, it is a stimulator for the central nervous system. Consumption of too much caffeine can be problematic, especially for teenagers and children. Therefore limits for caffeine in foodstuff exist, which are different in each country.

In Germany there should not be more than 0.32 mg/mL caffeine in any food or beverage. Children should not ingest more than 3 mg caffeine per kg bodyweight per day. For a 10 year old child with 30 kg bodyweight this is not more than 90 mg per day. Energy drinks often contain a high amount of caffeine, some of them even more than the country specific limit.

Thin layer chromatography (TLC) coupled with mass spectrometry (MS) is a straightforward and economic method to analyze caffeine in a complex matrix like an energy drink. It is not necessary to perform an elaborate sample preparation, samples can be applied undiluted onto the TLC plate. After chromatographic development the analyte can be extracted with a TLC-MS directly into the MS.

Results and Discussion

Caffeine can be detected under UV-light (254 nm) at an hRf value of 55 (Chromatographic data table on next page). The developed plate is shown in Fig. 1. Quantification was done using peak height after scanning the plate at 273 nm with the TLC Scanner. By doing a 4-point calibration the amount of caffeine in the samples was calculated. Thereby, values between 0.13 mg/mL and 0.41 mg/mL were determined (Tbl. 1). Therefore, two of the energy drinks have a caffeine value above the limit. Fig. 2a and b show the scan track at

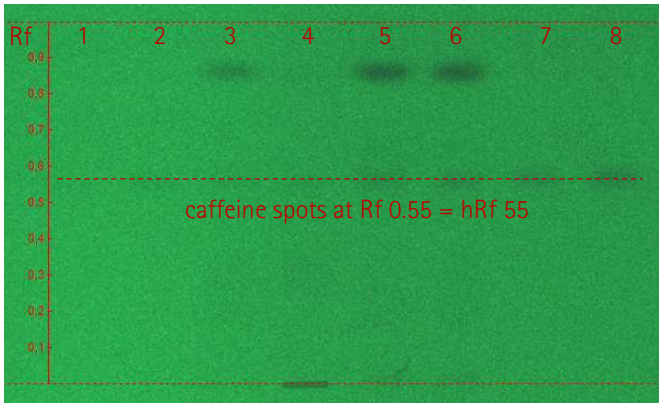

Fig. 1: HPTLC plate with caffeine standard and energy drink samples under UV light (254 nm).

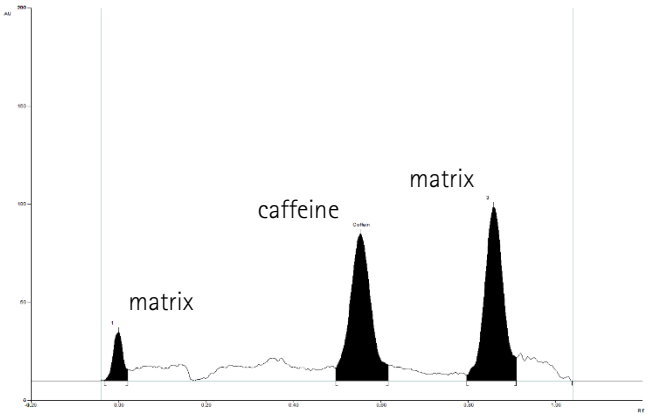

Fig. 2a: Scan of track 3 at 273 nm.

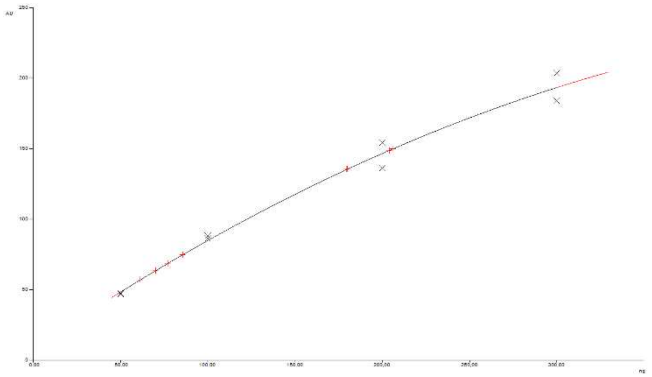

Fig. 2b: calibration curve of caffeine standards (track 1, 2, 7 and 8), black: standard, red: samples (two-fold determination).

273 nm and the related calibration curve. The mass of caffeine is clearly recognizable in the MS spectrum after background substraction. Thereby not only the mass of caffeine is visible but also an adduct of caffeine and acetonitrile (Fig. 3).

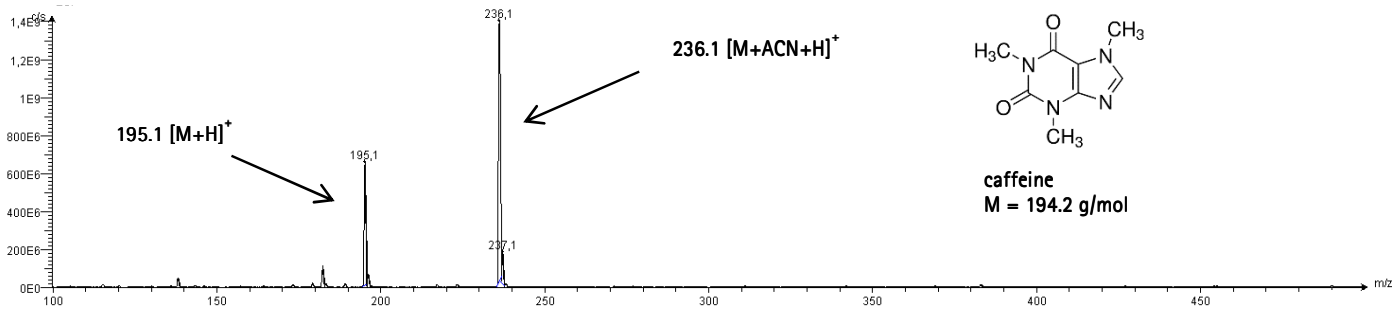

Fig. 3: mass spectrum of caffeine spot with background substraction.

Conclusion

Because of high matrix tolerance of TLC, complex samples like energy drinks can be analyzed quickly and without sample preparation. Quantification can be done with a TLC scanner. Using a TLC-MS Interface, TLC can be coupled with mass spectrometry and analytes can be detected by MS after separation. Therefore it is possible to get a complete solution for many analytical questions.

Determined caffeine values	
Energy drink 1	0.17 mg/mL
Energy drink 2	0.13 mg/mL
Energy drink 3	0.41 mg/mL
Energy drink 4	0.40 mg/mL

Tbl. 1: Results of caffeine analysis in energy drinks by quantification with TLC scanner from Camag. Limit for caffeine in beverages (Germany): 0.32 mg/mL.

Chromatographic data

Track	Compound	Conc. [mg/mL]	Application volume [µL]	hRf	Detected mass m/z
1	Caffeine standard	0.1	0.5	55	195.1
2	Caffeine standard	0.1	1.0	55	195.1
3	Energy drink 1	-	0.5	55	195.1
4	Energy drink 2	-	0.5	55	195.1
5	Energy drink 3	-	0.5	55	195.1
6	Energy drink 4	-	0.5	55	195.1
7	Caffeine standard	0.1	2.0	55	195.1
8	Caffeine standard	0.1	3.0	55	195.1

HPTLC Application number 407

Application data

Chromatography	Plate	HPTLC Silica gel 60 F_{254} MS-grade, 20x10 cm
	Sample preparation	no sample preparation
	Sample application	ATS 4 sample applicator (Camag), 6 mm bandwise
	Application volume	0.5 - 3 µL
	Mobile phase	2-Propanol / n-Heptane / Water 7/3/1 (v/v/v)
	Migration distance	5 cm
	Migration time	50 min
Extraction	Extraction equipment	"TLC-MS Interface" from Camag
	Extraction solvent	Acetonitrile / Water 95/5 (v/v) + 0.1% Formic acid
	Extraction flow	0.1 mL/min
Detection	Documentation equipment	documentation unit Reprostar / Digistore (Camag)
	Wavelength	scan under UV-light at 273 nm
	Staining	Anisaldehyde sulphuric acid reagent and heated 5 min at 120°C
	MS equipment	single-quadrupole mass spectrometer CMS (Advion)
	MS detection	ESI (+) mode MS (m/z 100 - 500)

Ordering information

HPTLC Silica gel 60 F_{254} MS-grade, 20x10 cm	1.00934
Acetonitrile hypergrade for LC-MS LiChrosolv®	1.00029
2-Propanol gradient grade for liquid chromatography LiChrosolv®	1.01040
n-Heptane gradient grade for liquid chromatography LiChrosolv®	1.04390
Formic acid for analysis EMSURE®	1.00264
Water for chromatography (LC-MS Grade) LiChrosolv®	1.15333