A-20.3

Assay control of trimethoprim and sulfamethoxazole isolated from cotrimoxazole

Key words

Qualitative and quantitative HPTLC - densitometry (absorbance) - antibiotics - trimethoprim - sulfamethoxazole - cotrimoxazole - quality control

Scope

Cotrimoxazole is a free name for the combination of the bactericidal trimethoprim and the microbicidal sulfamethoxazole in the ratio 1:5.

The method presented is suitable for the assay control of trimethoprim (TMP) and sulfamethoxazole (SMZ) isolated from cotrimoxazole tablets in mg/tablet. The permitted deviation of the declared content is ± 10%.

The active agents TMP and SMZ are transferred to a solution of chloroform and methanol which is chromatographed on HPTLC silica gel 60 F254. The agents are quantitatively determined by scanning by absorbance at 285 nm.

Advantages of using HPTLC for this analytical task

- Suitable for routine analysis with high sample throughput
- Suitable for quality control and stability tests
- Easy analysis method, readily transferable for similar applications

Chemicals

Chloroform
Methanol
Dichloromethane

Standards: trimethoprim, sulfamethoxazole

Sample preparation for assay control

1. Weigh 20 tablets separately and determine the mean tablet weight.
2. 20 tablets are pulverized.
3. Weigh in three amounts of about 50 mg of the powdered tablets in 100 mL measuring flasks and dissolve with 50 mL chloroform - methanol 1:1.
4. Sonicate the measuring flasks for 2-5 min in an ultra sonic bath and fill up to 100 mL with chloroform - methanol 1:1.
5. Particulate solutions need to be filtered before chromatography, e.g. through Whatmann filter GF/C.

Standard solution

8.0 mg TMP (trimethoprim) and 40 mg SMZ (sulfamethoxazole) are dissolved in chloroform - methanol 1:1 and filled up to 100 mL (TMP= 0.08 µg/µL, SMZ = 0.4 µg/µL).

Chromatogram layer

HPTLC silica gel Merck 60 F_{254}, 20x10 cm

Sample application

Spotwise with CAMAG Automatic TLC Sampler III, track distance 6 mm, distance from left edge 15 mm (= 29 tracks per plate), distance from lower edge 10 mm, delivery speed 50 nL/s.

Application pattern

Track	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	...
	S1	a	b	S2	c	a	S3	b	c	S4	...
µL	0.5	1.0	1.0	1.0	1.0	1.0	1.5	1.0	1.0	2.0	...
TMP ng/µL	40			80			120			160	...
SMZ ng/µL	200			400			600			800	...

Chromatography

In CAMAG twin trough chamber 20x10 cm with methanol - dichloromethane 2:8 with chamber saturation for 10 min, running distance 50 mm (about 9 min).

Densitometric evaluation

CAMAG TLC Scanner with CATS Software, scanning by absorbance at 285 nm with deuterium lamp, evaluation via peak area by polynomial regression.

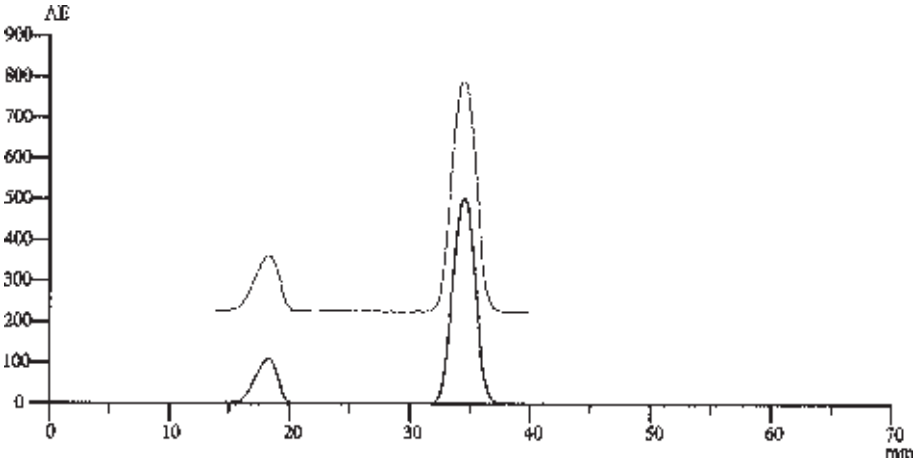

Figure 1: Densitogram of sample and standard

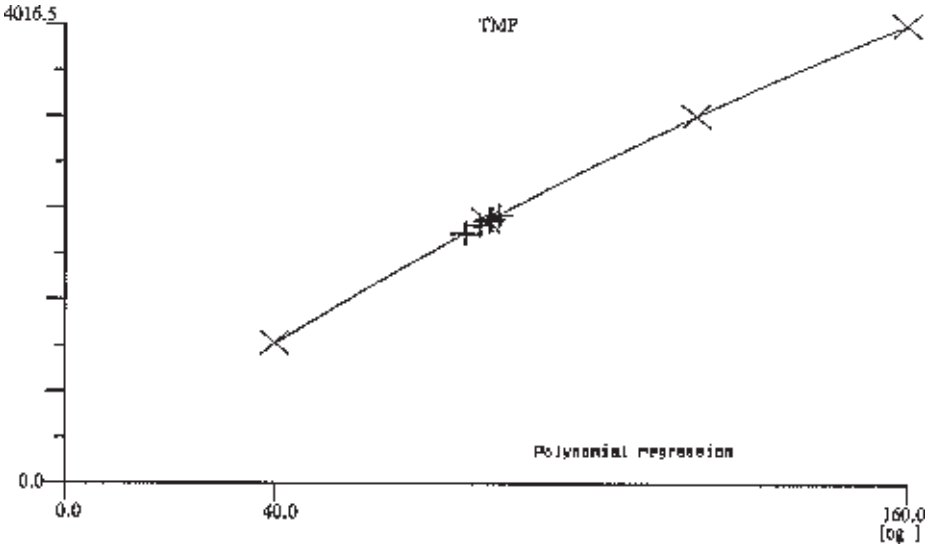

Figure 2: Calibration curve of TMP

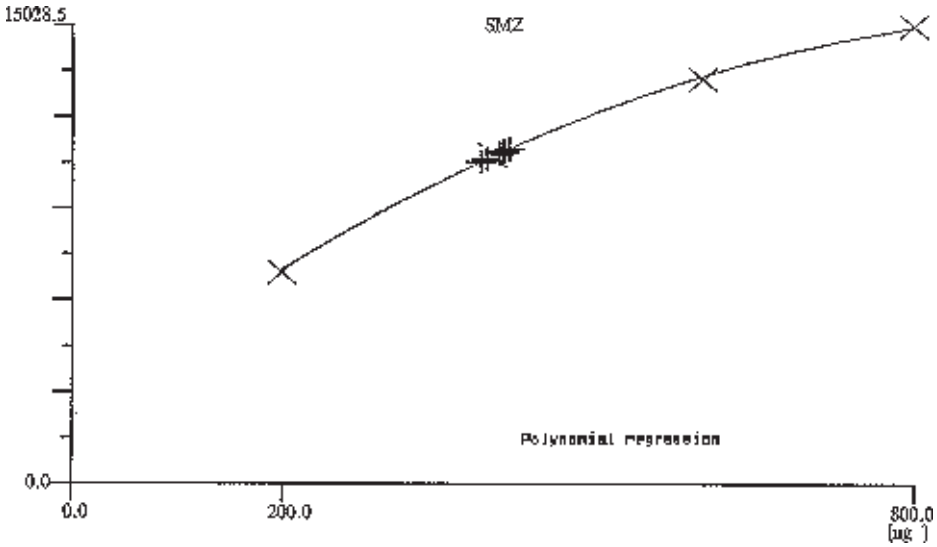

Figure 3: Calibration curve of SMZ

Results

In order to obtain results as mg/tablet, it is advisable to create the CATS method in calibration mode high complexity. Consequently all results are automatically calculated to the reference amount of, in this case, 1018 mg/tablet.

Sample	SMZ (800 mg/tabl.) mean value	VK [%] n=2	TPM (160 mg/tabl.) mean value	VK [%] n=2
a	782.132 mg/tabl.	0.97	154.771 mg/tabl.	2.6
b	821.224 mg/tabl.	0.74	162.244 mg/tabl.	0.26
c	830.434 mg/tabl.	0.93	164.406 mg/tabl.	1.2