Determination of trazodone via HPLC-SRM-MS

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I. BACKGROUND

Trazodone is an FDA-approved substance suitable for treating major depressive disorders. It is a serotonergic receptor antagonist as well as a serotonin reuptake inhibitor (SARI), which has better tolerance than second-generation SSRIs (selective-serotonin reuptake inhibitors). It can help to decrease inflammatory mediator concentration in neurons, thereby counteracting major depression. (Shin and Saadabadi 2018). In this experiment trazodone is determined in blood serum samples (n=3) via HPLC-SRM-MS. The samples are prepared by precipitation and centrifugation with clomipramine as an internal standard.

II. METHODS AND MATERIALS

A. MATERIALS

1) Instrumentation:

- HPLC (Agilent Technology 1260)
- ESI-SRM-MS (Agilent 6420 QqQ)
- column (Phenomenex Luna C18, 100 mm x 3 mm; 3 μm particle size.)

2) Chemicals:

- methanol (provided by the institue)
- milliQ water (provided by the institute)
- ammonium formate (provided by the institute)
- acetonitrile (provided by the institute)

B. METHODS

The calibration standards with increasing concentrations (see. Tab. II/c / mg L^{-1}]) are prepared by dilution of a stock solution (1 $\frac{mg}{ml}$) with 18 MΩ water. To correct for possible matrix effects or drag-out in the sample preparation, 50 $\frac{ng}{ml}$ of internal standard (clomipramine) are added. 50 μl of the internal standard are added to 100 μl of serum sample and proteins are precipitated by the addition of 350 μl methanol. The sample is homogenized using Thermoshaker ($\approx$ 5 min 1500 rpm). Furthermore the sample is centrifuged for 10 min at 4000 rpm. Subsequently 100 μl supernatant are diluted with 18 MΩ water (100 L sample solution + 900 μl 18 MΩ water) and analysed using LC QqQ (*Analytical Chemistry* 2019).

Time / min	A / %	B / %
0.00	70	30
1.00	70	30
10.00	40	60
10.01	70	30
13	70	30

TABLE I: The mobile phase A consisted of 5mM ammonium formate in H_2O, while eluent B contained acetonitrile.

The analytical run was set for 13 min, with a column temperature of 40 °C at 0,6 $\frac{ml}{min}$. The injection volume was 10 μl with a gradient displayed in Table I. The compounds of interest have the following $\frac{m}{z}$ ratios:
Trazodone: 372.2 (Precoursor) 148.0 (Product)
Clomipramine: 315.2 (Precoursor) 86.1 (Product)
The data was obtained via MassHunter, processed via R studio (V 1.2.1335) and visualized via the graphrobot webtool (R Core Team 2019, Wang 2019).

III. RESULTS

The calibration series of $c_{tradozone}$ against the ratio of the two Areas ($\frac{trazodone}{clomipramine}$) is provided in Table II and displayed in Fig. 1. The regression line contains the variables to the linear equation 1, with m = slope and t = intersept. For the following calculations x = analyte concentration and y = normalized peak area.

$$y = mx + t \tag{1}$$

The sample data (peak area) from Table III is used to determine the unknown concentration of the sample via Equation 1.

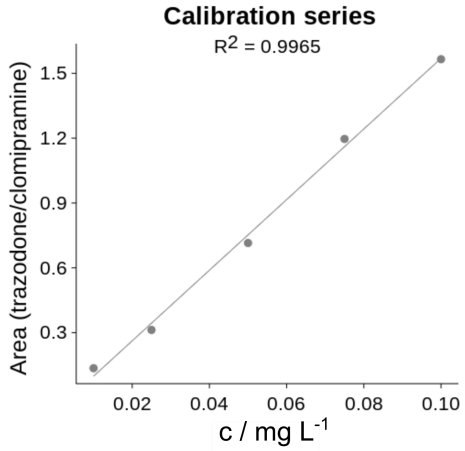

Fig. 1: External calibration of trazodone with an internal standard (clomipramine). The data for plot is derived from Table II. The regression equation has a Slope of 16.3399 and an Intercept of -0.0647

The results of the unknown concentrations give information about the diluted sample (see. Tab III/$c_{dil.}$ [c / mg L^{-1}]) and have to be multiplied by the dilution factor (50) in order to derive the sample concentrations: 2,21 3,15 and 2,56 ($\frac{\mu g}{ml}$) with a mean value of 2,64 $\pm$ 0,48 $\frac{\mu g}{ml}$ (Tab. III/ c / mg L^{-1}]).

c / mg L^{-1}	Ratio Area	trazodone		clomipramine	
		RT	Area	RT	Area
0,01	0,14	7,63	231687,14	1708973,46	3595,22
0,025	0,31	7,63	555189,95	1775912,61	7135,47
0,05	0,71	7,64	1293862,53	1809730,08	9066,31
0,075	1,20	7,64	2124889,82	1776068,70	6368,99
0,1	1,57	7,65	2644049,2	1689062,79	17189,34

TABLE II: Retention time (RT) and Area of external standard (trazodone c / mg L^{-1}]) and internal standard (clomipramine c = 50 $\frac{ng}{ml}$). Ratio area is calculated via $\frac{Area(trazodone)}{Area(clomipramine)}$

Sample						
trazodone		clomipramine		Ratio area	$c_{dil.}$ / mg L^{-1}	c / mg L^{-1}
RT	Area	RT	Area			
7,64	470522,96	715071,61	6472,36	0,66	0,04	2,21
7,64	392410,32	406244,40	3577,54	0,97	0,06	3,15
7,64	399391,40	517003,61	5556,86	0,77	0,05	2,56
						2,64 $\pm$ 0,48

TABLE III: Analysed samples (n = 3) with consequently calculated concentrations (c / mg L^{-1}]) via Equation 1, considering the dilution factor. Ratio area is calculated as in Tab. II.

IV. DISCUSSION

Peak plasma concentrations of trazodone after oral consumption occur approximately 1-2h. Plasma concentrations of trazodone decline in a biphasic manner. 100 mg of trazodone in healthy, fasted adults resulted in blood serum levels (c_{max}) 1.47 $\pm$ 0.16 $\frac{\mu g}{ml}$ (Kale and Agrawal 2015). The data does deviate strongly (+ $\approx$ 80 %) but can be assumed to be within a prescription based ingestion of up to 300 mg, if a linear context is presumed (Shin and Saadabadi 2018). Measurements of toxic blood serum levels are insufficient and controverse. As shown by Maria A. Martinez, nonfatal overdose blood concentrations of trazodone were as high as 30 $\frac{\mu g}{ml}$, with only mild symptoms of toxicity. Lethal trazodone blood concentrations in mixed overdoses have been measured from 9 to 32 $\frac{\mu g}{ml}$. However, only two fatalities were attributed to trazodone alone (Martínez et al. 2005). According to the presented sources, many variables (e.g age, sex, incubation time, feed state) influence the interpretation of the trazodone levels. Hence, no scientifically reasonable statement about the patients health state can provided.

V. APPENDIX

A. Experimental environment

The experiment was performed at TNF Turm JKU, Linz, Austria on 26.06.19 under the supervision of Armin Guntner.

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