Evaluation of a homogenous urine immunoassay for the determination of methadone in serum samples

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AIMS: The usefulness of plasma methadone concentration in dose adjustment of patients in maintenance treatment is still a matter of discussion. However, there are several other settings where a quick, cost effective and semiquantitative immunoassay for the determination of methadone in serum would be helpful. Besides intoxication and abuse screening verifying compliance of patients with take-home privilege gets more important. We therefore investigated if the methadone CEDIA urine assay can be adopted for serum level estimation and screening purposes.

METHODS: In total 267 serum samples from 203 patients with known dose of methadone (236 samples, 172 patients) or Polamidon (31 samples, 28 patients) were analysed. Dosing range for methadone was 15-280mg/d (mean 74mg/d) and for Polamidone 5-250mg/d (mean 50mg/d). Only patients in steady-state where trough level determination of methadone was requested were included. In addition 50 samples from 50 patients with no history of methadone use were analysed. Samples were analysed with CEDIA methadone and CEDIA Sample Check reagents according to the manufacturers urine application but with calibrators at 0, 50, 100, 300, 600 and 1000ng/mL and calibration using spline approximation. Instrument used was an Olympus AU640. Quantitative methadone determination was performed from 1mL serum after SPE (SPEC-dau, Varian) with isocratic HPLC-DAD on a 250/4 LiChrospher 100-5 RP8ec column (LOQ: 10ng/mL, linearity: 10-2000ng/mL). To better handle unforeseen co-elutions, the method was validated with two internal standards: maprotiline (200ng) and Chirald (500ng). A certified serum "replacement drugs"control was obtained from Medichem (Steinenbronn ; target value methadone: 107.5ng/mL).

RESULTS: There was only a weak correlation between methadone dose and serum concentration (range: 28-1305ng/mL). This can be explained with the well described inter-individual variability in metabolism but in this study had to be related to a certain extent also to noncompliant patients. When CEDIA values were correlated with methadone serum concentrations from HPLC, slope was 0.73 (intercept: 25.4, r^2: 0.83). Interestingly, Sample Check values representing CEDIA enzyme activity were reduced for all samples (mean: 85%, RSD: 2.7%). This matrix influence resulted in reduced methadone values esp. from the less sensitive ends of the sigmoidal calibration curve (<100ng/mL, >500ng/mL). False negative results were obtained from 11 samples (4.%, CEDIA: 0ng/mL) with corresponding HPLC values from 28-77ng/mL. Samples were reanalysed with both assays after 1:2 dilution with 0.9% saline, leading to improved Sample Check values (mean: 94%, RSD: 1.6%) and a slope of 0.97 (intercept: -3.7, r^2: 0.83) when CEDIA methadone values were compared to HPLC. Only 7 samples (2.6%, CEDIA 0ng/mL) remained false negative (HPLC: 28-66 ng/mL) and additional 51 (19%) were >5 and <100ng/mL (HPLC:27-173ng/mL). With this approach 20% of the samples were between 9% to 70% recovery and 11% were above 130%. From the samples below 70% recovery, 70% were <100ng/mL with CEDIA. To check CEDIA specificity 50 serum samples which did not contain methadone were analysed with and without dilution. All samples gave 0ng/mL with

methadone CEDIA and Sample Check means of 91% and 83% resp. The quality control serum used for HPLC (mean: 99.6ng/mL, RSD: 3.6%, n=30) was now applied to the CEDIA after 1:2 dilution: mean:102ng/mL (RSD: 7.7%), median: 101ng/mL, n=10.

CONCLUSIONS: The methadone CEDIA urine assay can be used for screening after 1:2 dilution and detects all serum concentrations >100ng/mL. A certain cutoff is not recommend, all samples with results >0ng/mL should be confirmed. In compliance testing, the lab has to be aware of false low CEDIA values. Nevertheless, it seems possible to support the suspicion of noncompliance to dosing from the CEDIA value if the patients previously determined individual steady-state level was much different. However, this steady-state level should have been determined with validated chromatographic methods.

KEYWORDS: *Serum methadone, Methadone CEDIA, Sample Check, Methadone compliance*

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